Culture de foraminifères benthiques profonds en conditions contrôlées : Calibration du δ18O en tant que proxy de paléotempérature.

Christine Barras

UNIVERSITÉ D'ANGERS

Année 2008
N° d'ordre : 943

Culture de foraminifères benthiques profonds en conditions contrôlées : Calibration du $\delta^{18}O$ en tant que proxy de paléotempérature.

THÈSE DE DOCTORAT
Spécialité : Sciences de la Terre et de l'Atmosphère
ÉCOLE DOCTORALE D'ANGERS

Présentée et soutenue publiquement
le 14 Novembre 2008
à Angers

par

CHRISTINE BARRAS

Devant le jury ci-dessous :

J.-C. Duplessy, DRCE Emérite, LSCE, Gif-sur-Yvette	Directeur de thèse
F. Jorissen, Pr., BIAF, Université d'Angers	Directeur de thèse
E. Geslin, MdC, BIAF, Université d'Angers	Co-encadrante
P. Heinz, MdC, Université de Tübingen, Allemagne	Rapporteur
T. Corrège, Pr., Université de Bordeaux 1	Rapporteur
J.-P. Gattuso, DR1, Laboratoire d'Océanographie, Villefranche-sur-Mer	Examinateur
G. J. Reichart, UD, Université d'Utrecht, Pays-Bas	Examinateur

BIAF
Laboratoire d'Etude
des Bio-Indicateurs Actuels et Fossiles
UFR Sciences - 2, bd Lavoisier
49045 Angers Cedex 01 - FRANCE

LSCE
Laboratoire des Sciences
du Climat et de l'Environnement
CNRS-CEA-UVSQ – Allée de la Terrace
91198 Gif-sur-Yvette Cedex - FRANCE

REMERCIEMENTS

TABLE DES MATIÈRES

INTRODUCTION

INTRODUCTION

PROXIES PALÉOCÉANOGRAPHIQUES BASÉS SUR LA CULTURE DE FORAMINIFÈRES BENTHIQUES PROFONDS EN CONDITIONS CONTROLÉES : CALIBRATION DU $\delta^{18}O$ EN FONCTION DE LA TEMPÉRATURE

Les foraminifères sont des organismes marins ubiquistes qui ont la particularité de construire leur coquille en enregistrant les conditions du milieu dans lequel ils vivent. La composition géochimique des coquilles carbonatées des foraminifères, qui présentent un fort potentiel de fossilisation, est donc largement étudiée afin de reconstituer les conditions océanographiques du passé. En particulier, la composition isotopique de l'oxygène des foraminifères fossiles est utilisée pour reconstituer le volume des glaces, la circulation des différentes masses d'eau, les paléo-températures et, combinée à d'autres outils tels que la composition en éléments traces (Mg/Ca), les paléo-salinités. Dans un premier temps, la calibration de cet outil a été basée sur des expériences en laboratoire en précipitant de la calcite inorganique dans différentes conditions de températures, mais aussi sur l'étude de foraminifères actuels (colorés au Rose Bengal) ou fossiles récents prélevés sur le terrain (calibrations *in situ*). Par la suite, la culture de foraminifères en laboratoire s'est développée, permettant de contrôler de manière précise les conditions de calcification et leur influence sur le $\delta^{18}O$ des coquilles de foraminifères. Jusqu'à présent, la majorité des expériences réalisées en laboratoire consistait à élever en culture des foraminifères planctoniques. En effet, ces organismes présentent l'avantage de calcifier rapidement suffisamment de calcite pour réaliser des mesures géochimiques. Toutes les études ont mis en évidence l'effet de la température et de la composition isotopique de l'eau, dans laquelle le foraminifère calcifie, sur la composition isotopique de sa coquille. Ainsi, plusieurs équations de paléo-température ont été établies permettant, à partir de la composition isotopique des coquilles de foraminifères et de celle de l'eau de mer, d'estimer la température de l'eau dans laquelle ils ont calcifié. D'autres effets secondaires, mais d'importance, ont aussi été identifiés tels que les effets vitaux spécifiques à chaque espèce et l'effet de la concentration en carbonate [CO_3^{2-}].

Actuellement, peu d'études se sont tournées vers la culture des foraminifères benthiques profonds en laboratoire. Plusieurs raisons peuvent être évoquées pour expliquer cette situation : (1) le temps et les moyens nécessaires pour la collecte d'échantillons contenant des foraminifères benthiques profonds vivants sont importants, (2) ces organismes pourraient avoir plus de difficultés à s'adapter aux conditions en laboratoire ; en effet, dans leur milieu naturel, leurs conditions de vie sont relativement stables en comparaison aux foraminifères planctoniques ou côtiers qui peuvent supporter des variations de température et salinité importantes ; leur collection implique qu'ils supportent les fortes variations de pression et de température lorsqu'ils sont ramenés en surface, (3) leur vitesse de calcification étant plus lente que celle des foraminifères planctoniques, la durée des expériences est par conséquent plus longue ; les conditions physico-chimiques dans le milieu de culture doivent donc être maintenues stables sur une plus longue période.
Mon travail de thèse s'inscrit dans ce contexte. L'intérêt général de cette thèse est de déterminer les conditions de culture en laboratoire des foraminifères benthiques profonds et d'étudier l'influence de la température sur la composition géochimique de leur coquille, le but final étant d'établir une équation de paléo-température.

D'après les expériences préliminaires réalisées avec des assemblages de foraminifères benthiques profonds du Golfe de Gascogne (échantillons prélevés à 450 et 650 m de profondeur), *Bulimina* a montré une bonne adaptation aux conditions en laboratoire. Par la suite, notre étude s'est donc concentrée sur ce genre. Après une introduction succincte sur la biologie, l'écologie et la géochimie des foraminifères ainsi que leur intérêt en culture, le **Chapitre 1** présente une discussion sur le sujet controversé de la classification en différentes espèces ou morphotypes de *Bulimina*. En effet, certaines études considèrent *B. marginata* et *B. aculeata* comme deux espèces distinctes tandis que d'autres soutiennent l'hypothèse de l'existence d'une seule espèce, *B. marginata*, possédant différents morphotypes, avec notamment un morphotype *marginata* et un morphotype *aculeata*. Sur la base de l'analyse des principaux articles disponibles dans la littérature traitant de la taxonomie, de l'écologie et de la composition géochimique de ces deux « espèces », nous avons pris un choix pragmatique, sans pour autant résoudre cette question polémique.

Afin de réaliser des expériences dans des conditions géochimiques contrôlées, il a été dans un premier temps primordial de définir les conditions optimales de vie de *Bulimina marginata* en laboratoire (**Chapitre 2**). Différentes conditions de température et de nourriture ont été testées

afin d'étudier leur influence sur les caractéristiques biologiques de cette espèce, en particulier, sur la reproduction (temps nécessaire avant d'observer la première reproduction, nombre de juvéniles produits par reproduction…) et la croissance (taux de croissance de la coquille).

Une deuxième phase du travail a consisté à mettre au point les protocoles expérimentaux permettant d'élever ces foraminifères benthiques profonds dans des conditions stables et contrôlées (**Chapitre 3**). Deux systèmes sont décrits dans ce chapitre : (1) un système fermé, « closed system », qui consiste à faire circuler une importante quantité d'eau à travers différentes bouteilles contenant les foraminifères et leur nourriture, et (2) un système élaboré en boîte de Pétri, « Petri dish system », où l'eau de mer des boîtes de Pétri où vivent les foraminifères est changée régulièrement de manière à éviter toute évaporation ou diminution de pH. La variabilité des différents paramètres physico-chimiques (température, salinité, pH, alcalinité) pouvant influencer la composition isotopique des coquilles de foraminifères est présentée et analysée.

Enfin, nous avons étudié la composition isotopique en oxygène et en carbone d'individus de *Bulimina marginata* (*sensu lato*) ayant calcifié leur coquille à différentes températures dans les systèmes précédemment décrits (**Chapitre 4**). Les données ont permis d'évaluer l'importance des effets biologiques sur la teneur en isotopes stables pour cette espèce, en particulier l'effet de la taille. Les équations liant la température au $\delta^{18}O$ des coquilles ont été établies en fonction de la classe de taille considérée. Ces équations de calibration ont pu être comparées à l'équation obtenue à partir d'individus prélevés sur le terrain.

Dans une dernière partie, nous présentons une synthèse des données qui ont été obtenues au cours de cette étude et indiquons des perspectives pour de futures recherches.

CHAPITRE 1

STATE OF THE ART

CHAPITRE 1

STATE OF THE ART

1. WHAT IS A FORAMINIFER?

According to the classification of Loeblich and Tappan (1964), foraminifera are single-celled protozoa, which belong to the Kingdom Protista and the Class Granuloreticulosea. Depending on the species, their shell (also called tests) may be organic, agglutinated (mineral grains cemented together), composed of calcium carbonate (biomineralisation of calcite or aragonite) or, in rare cases, in silica.

In practice, foraminifera are divided into two groups based on their mode of life, planktonic (living in the water column) and benthic (living in contact with the sediment). Benthic foraminiferal shells can be composed of one single chamber or divided into chambers which are added intermittently during growth. In the latter case, for some species, a layer of calcite is deposited over both the new chamber and over the entire test. Generally, individuals range in size from 38 µm to 1 cm long (Murray, 2006) although larger taxa do also exist (up to 2 cm).

The morphology of the test, from the microstructures to the shape and chamber arrangement, is the main criterium of the actual classification of foraminifera (Loeblich and Tappan, 1988; Sen Gupta, 1999b). Since these classifications do not take into account genetic analyses, foraminiferal species are for the moment considered as morphological taxa based on a phenetic classification. The increasing number of studies focussing on molecular phylogenetic analyses based on rDNA sequences may ultimately lead to a significant modernization of the classification of foraminifera (e.g. Pawlowski, 2000; Pawlowski and Holzmann, 2002; Schweizer *et al*., 2005, 2008).

2. BIOLOGY OF BENTHIC FORAMINIFERA

Foraminifera possess a cytoplasm which presents the particularity to extend outside of the test through single or multiple apertures and test perforations. These thin extensions, called pseudopodia, accomplish many essential life functions: motility (e.g. migration in fine-

grained sediments; Hemleben and Kitazato, 1995), attachment, feeding, building and structuring tests, and protection (Figure 1.1). With the pseudopodia, foraminifera are able to construct a sort of cyst by gathering algal cells, bacteria, and organic detritus. These cysts have been observed to be used for feeding, or to appear before the formation of new chambers or reproduction (e.g. Goldstein and Corliss, 1994; Debenay *et al*., 2000; Heinz *et al*., 2005).

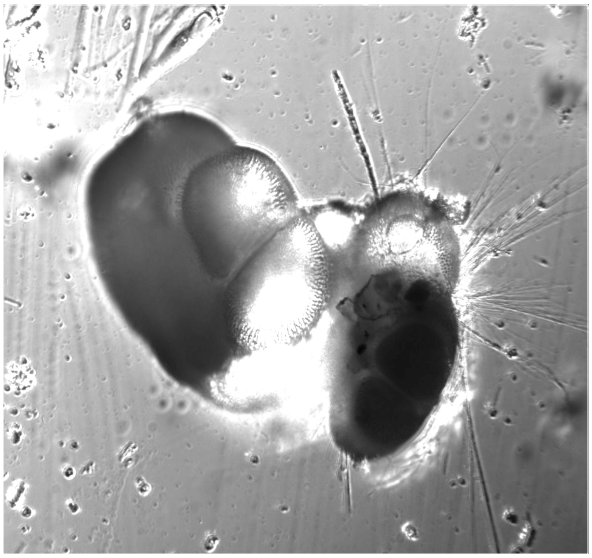


Figure 1.1: Picture of a reticulopodial net extended by Ammonia tepida *(pictured by Sandra Langezaal Utrecht University).*

.

Benthic foraminifera are heterotrophic organisms. Consequently, they require organic compounds to obtain the carbon essential for their metabolism (growth, reproduction, locomotion; Myers, 1943). In general, Foraminifera utilize a broad range of feeding mechanisms and nutritional resources, including grazing, suspension feeding, deposit feeding, carnivory, parasitism, direct uptake of Dissolved Organic Carbon, and symbiosis (Goldstein, 1999). Some species of foraminifera are living in symbiosis with algae (Lee and Anderson, 1991; Lee *et al*., 1995) or with bacteria (Bernhard, 2003).

In the deep sea (deeper than 200 m water depth, representing the limit between the continental shelf and the continental slope), benthic organisms are mainly depending on the phytodetritus flux to the sea floor which in turn depends on the primary production in the photic zone of the water column. More degraded forms of organic detritus, and also the large bacterial community associated with this material, may also provide important food resources for specimens living in the sediment (Goldstein and Corliss, 1994).

The life cycles of benthic foraminifera have been studied for about 30 species (Lee *et al*., 1991; Goldstein, 1999). Foraminifera present a life cycle characterised by an alternation of sexual and asexual generations (full arrows, Figure 1.2).

By gametogenesis, the adult gamont produces haploid gametes, which are directly discharged into the surrounding seawater. Fertilization takes place by the fusion of two gametes, resulting in a zygote (first diploid cell produced by sexual reproduction). After growth, the diploid, multinucleate adult agamont obtained reproduces asexually. Meiosis occurs in the agamont as part of multiple fission. The meiosis is a reduction division process: the diploid number of chromosomes per cell is halved and the daughter cells produced are haploid. Therefore, the resulting young become haploid, uninucleate adult gamonts (Murray, 2006).

Alternatively, in some species, the life cycle includes successive asexual cycles (dashed arrows, Figure 1.2). In this case, the agamont may produce, instead of a gamont, a second asexual generation, the schizont. This individual may produce a number of successive asexual generations by schizogony (multiple fission of the nucleus followed by the segmentation of the cytoplasm). The type of nuclear divisions that occurs in schizonts has not been documented. This facultative alternation of generations is referred to as biological trimorphism (reviews in Lee *et al*., 1991; Goldstein, 1999).

It should be noted that for a single species, these two or three generations may be morphologically significantly different. This had lead to considerable confusion in foraminiferal taxonomy. The size of the proloculus (initial chamber) varies systematically between the different generations. Since the asexually produced haploid generation commonly forms a large proloculus, they are termed megalospheric. Sexually produced diploid generations tend to produce a smaller proloculus and are therefore termed microspheric. Most of the time, adults die after the release of the gametes or of the juveniles (Murray, 2006).

Although still badly known, it is estimated that the duration of life of foraminifera can range from 3 months up to 2 years (Murray, 1983; Hallock *et al*., 1986).

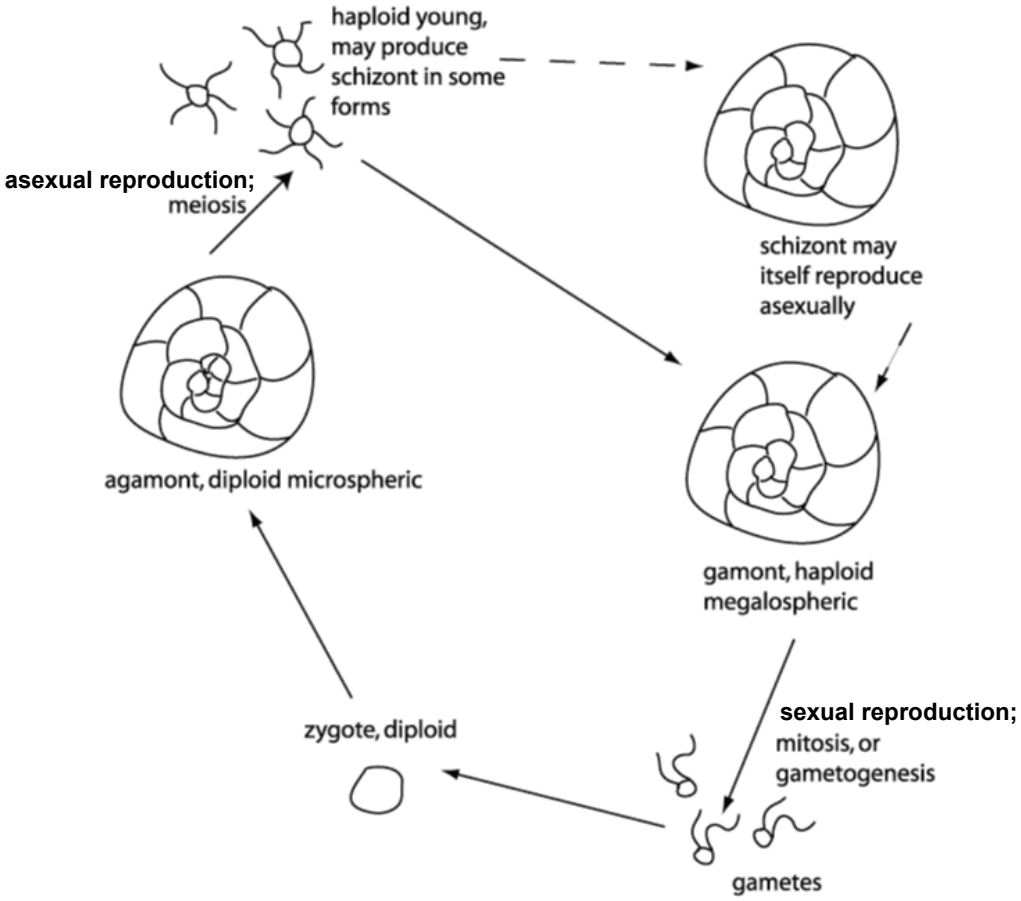


Figure 1.2: Diagram showing a generalised foraminifera life cycle. Note the alternation between a haploid megalospheric form and a diploid microspheric form (redraw from Goldstein, 1999).

3. ECOLOGY OF BENTHIC FORAMINIFERA

Benthic foraminifera are ubiquist (from brackish estuaries to the deep ocean basins and at all latitudes), present a wide diversity in marine environments, and have a high fossilisation potential (Murray, 2006). Due to their relatively short life span, foraminifera are able to adapt and response rapidly to environmental changes. These characteristics make them good bioindicators of environmental changes in modern and past Oceans. Most foraminifera are limited to marine environments, and 99% of foraminiferal taxa are benthic.

3.1. Parameters influencing benthic foraminiferal distribution

Different parameters have an impact on the density, composition and microhabitat (vertical distribution in the sediment) of benthic foraminifera, e.g. organic matter input, bottom and pore water oxygenation, temperature, salinity, particle size of the sediment and predation.

Today, it is thought that in deep-sea environments, the main parameter structuring benthic foraminiferal faunas is the organic matter (quality, quantity and periodicity) flux exported to the sea floor (e.g. Lutze and Coulbourn, 1984; Altenbach and Sarnthein 1989; Corliss, 1991; Herguera and Berger 1991; Rathburn and Corliss, 1994; Levin and Gage, 1998; Jorissen *et al*. 1998; De Rijk *et al*., 2000; Fontanier *et al*., 2002; Licari *et al*., 2003; review in Jorissen *et al*., 2007). For example, episodic phytoplankton bloom events, and the subsequent deposits of the algal remains on the sea floor, may provoke a very strong reproduction and growth response of deep-sea benthic foraminiferal species.
Since the early 1990s, studies have reported that several foraminiferal species can live, and be active, in anoxic sediments (e.g. Bernhard and Reimers, 1991; reviews in Bernhard and Sen Gupta, 1999; Jorissen *et al*., 2007). Therefore, oxygen concentration of bottom and interstitial waters can act as a limiting factor only below concentrations of 1 $ml.l^{-1}$ or less (e.g. Sen-Gupta and Machain-Castillo, 1993; Levin and Gage, 1998; Gooday *et al*., 2000).

According to van der Zwaan *et al*. (1999), oxygen would control the specific richness of foraminiferal faunas whereas food availability would control their density. However, these two parameters are interdependent and negatively correlated, i.e. the degradation of a high quantity of organic matter consumes oxygen. Consequently, eutrophic areas (with high organic carbon input) are often oxygen depleted, whereas oligotrophic areas (low organic carbon input) are generally well oxygenated. Therefore, it is very difficult to disentangle the two signals and to determine how each of these two factors controls the benthic foraminiferal faunas.

3.2. Microhabitat

The microhabitat indicates the foraminiferal distribution in the topmost sediment layer. Foraminifera are usually restricted to the upper 5 to 10 cm. This distribution is the result of the physico-chemical conditions and the biological activity in the sediment, which in turn is

directly linked to the organic matter flux and the oxygenation conditions (e.g. Shirayama, 1984; Corliss and Emerson, 1990; Jorissen *et al*., 1995), but also other factors such as interspecific competition (Gooday, 1986).
When the exported organic matter reaches the ocean floor, its degradation induces numerous biogeochemical reactions in the sediment. These processes are known as "early diagenesis" (e.g. Froelich *et al*., 1979, Anschutz *et al*., 2000; Hyacinthe *et al*., 2001). In the surficial sediment, the oxidation of the organic matter is due to aerobic processes; the oxygen is the foremost acceptor of electrons, which has the highest energetic yield. When oxygen is no longer available (below the zero oxygen level), organic matter degradation processes are based on other oxidants: nitrates, manganese and iron oxides, and sulphates. These processes involve a large diversity of bacteria. Although the exact pathways are not yet fully understood, it is clear that the vertical distribution of foraminifera in the sediment depends on this succession of biogeochemical reactions, especially below the oxygen zero boundary. This succession of biogeochemical reactions is itself dependent on the organic matter input.

Different types of microhabitat are identified. Species living at the sediment surface (e.g. *Cibicides*/*Cibicidoides*) have an epifaunal microhabitat. In the sediment, we distinguish between: shallow infaunal species (e.g. *Uvigerina*), living in the upper part of the oxic zone, intermediate infaunal species (e.g. *Melonis barleeanus*), generally living in the lower part of the oxic zone, and deep infaunal species (e.g. *Globobulimina*) living preferentially around the zero oxygen limit and in anoxic sediment. Some species capable to live in totally anoxic sediment are able to stock nitrates and use them as energetic sources, such as was discovered by Risgaard-Petersen *et al*. (2006) for *Globobulimina* spp.. Finally, some species exhibit no preferential microhabitat and can be found throughout the sediment with a maximum of density at the sediment surface and also one deeper in the sediment (e.g. *Bulimina,* Jorissen, 1999a).

A conceptual model, the so-called TROX model, has been proposed by Jorissen *et al*. (1995) to explain the foraminiferal distribution according to different trophic and oxygen conditions (Figure 1.3). According to this model, the oxygen penetration depth determines the living depth of organisms, and if oxygen is not limiting, the foraminiferal distribution is determined by the food availability. In other words, in oligotrophic areas, the labile organic matter is quickly metabolised in surface and the sediment is well oxygenated until deep layers. In this case, the limiting factor is the food availability, so these environments are dominated by

epifauna and shallow infaunal taxa, whereas intermediate and deep infaunal taxa are rare. In eutrophic environments, the organic matter flux and the oxygen consumption are high. Generally, the oxygen is depleted just below the sediment-water interface. Therefore faunas are mainly present close to the sediment surface. Deep infaunal species, which are tolerant to dysoxia, may dominate the faunas in such settings. In intermediate conditions (mesotrophic environments), a well established succession of shallow, intermediate and deep infaunal microhabitats may be found in the sediment. These distribution patterns have been confirmed by numerous studies in deep-sea environments (e.g. Corliss, 1985, 1991; Barmawidjaja *et al*., 1992; Buzas *et al*., 1993; Schmiedl *et al*., 2000; Fontanier *et al*., 2002; Heinz and Hemleben, 2003; Koho *et al*., 2007; Schumacher *et al*., 2007).

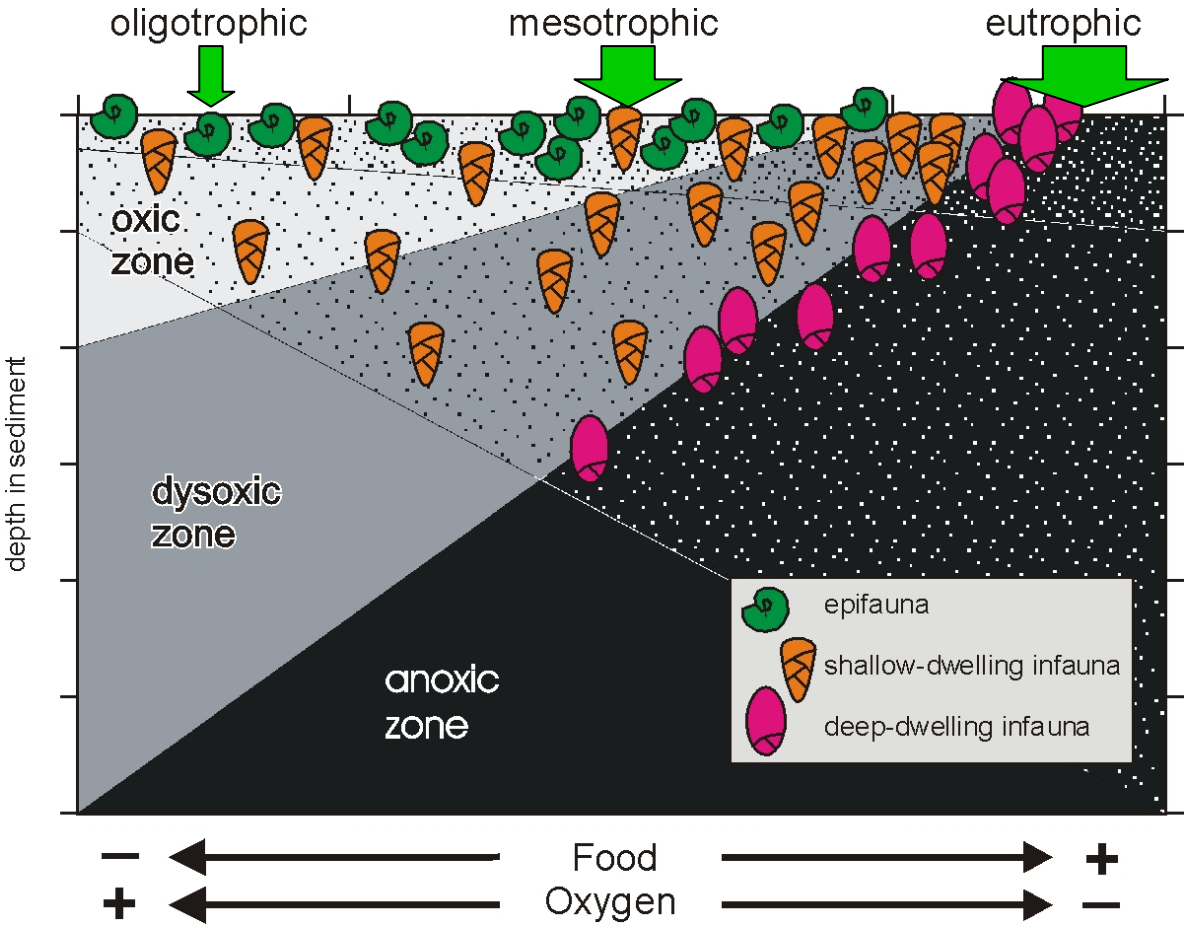


Figure 1.3: Microhabitat model in function of the oxygen penetration depth and the organic matter input (Jorissen et al.*, 1995; de Stigter, 1996).*

4. STABLE ISOTOPES: PROXIES FOR PALEOCEANOGRAPHIC STUDIES

Calcareous benthic foraminifera have the particularity to record in their test the environmental conditions in which they calcify. Because of their high fossilisation potential and their appearance from the Cambrian until the Present (Sen Gupta, 1999a), these organisms are widely used as tools (or proxies) for paleoceanographic reconstructions.

Since several decades, the oxygen and carbon stable isotopic composition are measured in calcareous foraminiferal tests. There are three stable isotopes of oxygen: ^{16}O, ^{17}O and ^{18}O (with relative natural abundances of 99.76, 0.04 and 0.20%, respectively), and two stable isotopes of carbon: ^{12}C (98.89%) and ^{13}C (1.11%). Fractionation (partitioning of isotopes between substances during physical, chemical or biological processes) essentially depends on thermodynamic laws ("equilibrium isotope fractionation") (Urey, 1947), but may be significantly influenced by biological processes. Basically, molecules consisting of light isotopes are more reactive than those consisting of heavy isotopes.

The isotopic ratios $^{18}O/^{16}O$ and $^{13}C/^{12}C$ (R) in carbonates are measured with a mass spectrometer. Foraminiferal shells are dissolved with phosphoric acid (H_3PO_4). The released carbon dioxide is purified under vacuum and is then introduced to the source of the mass-spectrometer where the following masses are measured: 44 ($^{12}C^{16}O^{16}O$), 45 ($^{13}C^{16}O^{16}O$) and 46 ($^{12}C^{18}O^{16}O$). Because absolute abundances of minor isotopes (^{18}O or ^{13}C) cannot be determined accurately, the signal of the sample obtained is compared to the one of a standard $CO_{2(gas)}$ of known isotopic composition. Masses ratios 45/44 and 46/44 allow to measure respectively $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios. These differences in isotope ratios are defined as:

$$\delta\,(‰) = [(R_{sample}-R_{standard})/\,R_{standard}] \times 1000$$

The standard for both oxygen and carbon in carbonates is referred to as PDB (Pee Dee Belemnite), having $\delta^{18}O=0$ and $\delta^{13}C=0$ by definition (Epstein *et al*., 1953). A positive δ value indicates enrichment in the heavy isotope, relative to the standard, and inversely, a negative value indicates depletion.

4.1. Oxygen stable isotopes

The oxygen isotopic composition of calcite secreted by organisms depends primarily on the $\delta^{18}O$ of the seawater in which they calcify and on the temperature. This is due to the fact that fractionation of oxygen isotopes between carbonate and water is temperature dependent (Urey, 1947; Shackleton and Opdyke, 1973).

The seawater $^{18}O/^{16}O$ ratio is altered whenever water undergoes a phase change. Therefore, seawater $\delta^{18}O$ is intimately linked with the hydrological cycle: evaporation, atmospheric vapour transport, and return of freshwater to the ocean via precipitation and runoff, or iceberg melting (review in Rohling and Cooke, 1999). For example, when seawater evaporates, the heavier isotope ^{18}O is preferentially left behind in remaining seawater, while the resulting water vapour is depleted in ^{18}O. Seasonal sea ice formation and melting impose strong local variability. Melting of sea ice (low $\delta^{18}O$ composition) results in the relative depletion of the seawater receiving this freshwater input. Therefore, the oxygen isotopic composition of seawater is also closely linked to salinity. The $\delta^{18}O$ of seawater is expressed in δ with respect to the ratio of Standard Mean Ocean Water (SMOW). In this study, we applied the conversion factor established by Hut (1987) to convert values from SMOW to PDB (-0.27‰).

The precipitation of calcium carbonate is governed by thermodynamic reactions so that the oxygen isotopic composition of carbonates could be used as a paleothermometer. In fact, foraminifera use dissolved ions from the seawater (CO_2, HCO_3^-, CO_3^{2-}) to biomineralise their test. Carbonate ions containing ^{18}O show a different precipitation rate than the carbonate ions containing light ^{16}O and this rate is function of the temperature. The relationship between $\delta^{18}O$ and temperature was studied by several authors in order to establish paleotemperature equations derived from inorganically precipitated calcite (McCrea, 1950; O'Neil *et al*., 1969; Kim and O'Neil, 1997), mollusc shells (Epstein *et al*., 1953; Horibe and Oba, 1972) or foraminiferal shells (Emiliani, 1955; Shackleton, 1974; Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Bouvier-Soumagnac *et al*., 1986; Bemis *et al*., 1998). In particular, the temperature dependency of benthic and planktonic foraminiferal isotopic fractionation was determined either from field samples (core tops, sediment traps, multinets) or from laboratory experiments with planktonic foraminifera. To our knowledge, no calibration based on laboratory cultures of benthic foraminifera, and especially deep-sea benthic foraminifera, has been undertaken until today. In general, the temperature effect with

respect to equilibrium fractionation is roughly 0.20-0.25‰ per 1°C, depending on the paleotemperature equation. In our study, we will compare our data to the paleotemperature equation defined by Shackleton (1974), adapted for deep-sea benthic foraminifera from the equation of O'Neil *et al*. (1969) for inorganic calcite:

$$T = 16.9 - 4.38\ (\delta^{18}O_f - \delta^{18}O_w) + 0.10\ (\delta^{18}O_f - \delta^{18}O_w)^2$$

4.2. Carbon stable isotopes

Shackleton (1977) showed the potential significance of $\delta^{13}C$ variations in studying paleoproductivity and water mass movement. The carbon isotopic composition of benthic foraminiferal shell depends on the $\delta^{13}C$ composition of bottom water, which is in turn directly linked to the organic matter input to the sea floor. In the surface waters, phytoplankton is enriched in light carbon isotope because of photosynthesis which is strongly discriminative in favour of ^{12}C. When the exported organic matter reaches the seafloor, its remineralisation releases depleted $\delta^{13}C$ into bottom water (depleted $\delta^{13}C_{DIC}$). Generally, increased primary production in surface water results in increased decomposition of organic matter in the sediment and consequently the benthic foraminiferal test is more depleted in ^{13}C. Usually, the $\delta^{13}C$ of carbonates is lighter if the quantity of remineralised organic matter is high.
The $\delta^{13}C$ values of benthic foraminiferal test also records water masses ventilation, therefore allowing to determine the respective ages of the water masses. Before sinking, deep waters present similar composition to that of the surface waters from where they originate (enriched in ^{13}C). Older deep waters which are more distal from their source have a relatively lighter composition due to their isolation from the atmosphere.

4.3. Vital effects

When thermodynamic relationships are the only factors affecting the fractionation of isotopes during precipitation of $CaCO_3$ from solution, the isotopes are precipitated in equilibrium (as described before). However, in many cases, deviations from equilibrium of oxygen and carbon isotopes are observed in biogenic carbonates. The poorly understood biological processes responsible for this disequilibrium, with different species which fractionate isotopes

somewhat differently, are known as "vital effects". It is thought that the main processes involved in these vital effects are kinetic and metabolic effects.

Kinetic effects result from the discrimination against heavy carbon and oxygen isotopes during the hydration and hydroxylation of CO_2. The exchanges during these reactions are slower than the precipitation of CO_3^{2-} (McConnaughey, 1989a, 1989b). Rapid calcification rates would result in more depleted isotopic composition of foraminiferal shell because precipitation would occur before equilibrium is achieved (Erez, 1978; McConnaughey, 1989a, 1989b). **Metabolic effects** result from changes in $\delta^{13}C$ and $\delta^{18}O$ of CO_2 and HCO_3^- in the region of the calcium carbonate precipitate, due to biological processes such as respiration or photosynthesis (McConnaughey, 1989a). For example, respiration results in $\delta^{13}C$ depletion of dissolved inorganic carbon and therefore a decrease in shell $\delta^{13}C$ (addition of light ^{12}C into the environment from where the $CaCO_3$ precipitates).

The effect of the size of shells on the isotopic composition of calcifying organisms such as corals and foraminifera is called **ontogenetic effect**. The ontogenetic effect has been studied in planktonic foraminifera showing an increase in stable isotope values with test size (e.g., Berger *et al*., 1978; Spero and Lea, 1996; Bijma *et al*., 1998). Younger specimens calcify faster and their metabolic activity is more intense so that both kinetic and metabolic effects lead to lighter isotopic composition of the shell (e.g. Berger *et al*., 1978; Turner *et al*., 1982). In contrast, similar trends have not been observed systematically in deep-sea benthic foraminifera (e.g., Vincent *et al*., 1981, Wefer and Berger, 1991; Corliss *et al*., 2002). The only exceptions reported so far include the taxa *Uvigerina mediterranea* and *Uvigerina peregrina* (Dunbar and Wefer, 1984; Schmiedl *et al*., 2004).

More recently, Spero *et al*. (1997) and Zeebe (1999) identified another vital effect responsible for isotopic disequilibrium: the **carbonate ion effect**. In laboratory experiments with planktonic foraminifera, Spero *et al*. (1997) observed that $\delta^{18}O$ and $\delta^{13}C$ in foraminiferal carbonate decreases with increasing $[CO_3^{2-}]$, the magnitude of the impact depending on the species considered. The authors suggested that kinetic fractionation was responsible since CO_2 hydration and hydroxylation depend on pH. According to Zeebe (1999), the explanation would reside in the fact that the different carbonate species have different equilibrium fractionations with respect to seawater and the relative abundance of these carbonate species varies as a function of pH. Assuming that calcium carbonate is formed from a mixture of the

carbonate species in proportion to their relative contribution to dissolved inorganic carbon, with increasing pH, the solution would consist of relatively higher concentrations of CO_3^{2-} (isotopically lighter than HCO_3^-) and therefore carbonate shell would be depleted in heavy oxygen and carbon isotopes.

In addition, studies on living benthic foraminifera from above and within the sediment indicated strong **microhabitat effects** on the stable isotope composition, of carbon solely, of different taxa (e.g. McCorkle *et al*., 1985, 1990, 1997; Rathburn *et al*., 1996; Mackensen *et al*., 2000; Corliss *et al*., 2002; Mackensen and Licari, 2004; Schmiedl *et al*., 2004). Usually, the profile of ambient pore water $\delta^{13}C$ shows a rapid isotopic depletion with depth in the sediment, caused by the decomposition of isotopically light sedimentary organic matter (e.g. McCorkle *et al*., 1985). Infaunal taxa would not calcify in equilibrium with bottom water $\delta^{13}C$ but with pore water $\delta^{13}C$. Therefore, the $^{13}C/^{12}C$ ratio of epifaunal species is enriched compare to the one of infaunal species.

Two other vital effects are documented in the literature, the gametogenetic calcite effect and symbiont photosynthesis effect. However, deep-sea benthic foraminifera are not concerned by them so we will not further develop these effects here.

5. LABORATORY CULTURE EXPERIMENTS

The complexity of factors influencing the spatial and temporal distribution of benthic foraminifera in the ocean and the stable isotopic composition of their shell demonstrates the necessity to perform laboratory experiments with living specimens. Cultures offer a new way to better understand the biology and the geochemistry of foraminifera.

Since the beginning of the 1940s, researchers perform laboratory experiments on foraminifera to observe their biological activities, e.g. movements, growth and reproduction (e.g. Myers, 1935; Arnold, 1954; Angell, 1990; Hemleben and Kitazato, 1995; Stouff *et al*., 1999a; Gross, 2000; Pawlowski *et al*., 1995). For example, the major role of foraminiferal pseudopodes for nutrition and motility was observed mainly in culture experiments. Also, the reproduction cycles were determined in culture, since for a long time, observations in the field were not possible (review in Lee *et al*., 1991). The interest of laboratory studies is also the possibility to test the impact of abiotic parameters on foraminiferal behaviour. For example, Bradshaw

(1957, 1961) studied the survival, growth and reproduction response of the shallow benthic foraminifera *Ammonia beccarii tepida* to different conditions of temperature and salinity. Moreover, culture experiments allow to determine the influence of a single parameter while the others are kept constant. In the field, it is often difficult or impossible to differentiate the effect of different, often interdependent, parameters. A good example of this problem is encountered when studying the influence of oxygen and food conditions. As we explained before, the concentration of oxygen and the quantity of organic matter are inversely linked. Several studies tried to understand the response of foraminifera to varying oxygen conditions (e.g. Alve and Bernhard, 1995; Geslin *et al*., 2004) or to different food input (e.g. Heinz *et al*., 2001, 2002; Ernst *et al*., 2004, 2005; Nomaki *et al*., 2005a, 2006).

The basic technical means necessary to study the behaviour of foraminifera in laboratory conditions are a stereomicroscope, video, scanning electron microscope (SEM) and coloration methods. Until today, the Rose Bengal coloration probe has most often been used to distinguish living from dead specimens. Recently, also non lethal probes, such as CellTracker Green or Calcein, have been applied to foraminifera (e.g. Bernhard, 2000; Bernhard *et al*., 2004, 2006). With the development of new technologies and new probes, researchers try to establish in detail the biomineralisation processes involved in foraminifera (Bentov and Erez, 2005; Toyofuku *et al*., 2008). For example, Toyofuku *et al*. (2008) studied the calcium ion movement in foraminiferal cells using a fluorescent probe.

Nowadays, the new interest of laboratory experiments with foraminifera is to study the geochemical composition of their test (e.g. $\delta^{18}O$, $\delta^{13}C$, Mg/Ca, Sr/Ca) according to different physico-chemical conditions (e.g. temperature, salinity, carbonate ion concentration), with the aim to better calibrate these proxies for paleoceanographic reconstructions. Most of these geochemical studies were performed with planktonic foraminifera, which present the advantage that they calcify rapidly enough calcium carbonate for isotope or trace element measurements (e.g. Bemis *et al*., 1998; Spero and Lea, 1996; Russell *et al*., 2004). For the same purpose, only few experimental studies with benthic foraminifera have been performed until now (Chandler *et al*., 1996; Wilson-Finelli *et al*., 1998; Toyofuku *et al*., 2000; Hintz *et al*., 2004, 2006a, 2006b). The most successful laboratory experiments have been performed with shallow benthic foraminifera, such as *Ammonia beccarii* and *Ammonia tepida* (e.g. Bradshaw, 1955, 1957, 1961; Stouff *et al*., 1999a, 1999b, 1999c; Le Cadre *et al*., 2006; Toyofuku *et al*., 2008). At the present time, experiments to better calibrate paleoceanographic proxies are performed with *Ammonia* in the Alfred Wagner Institüt in Bremerhaven and in the

laboratory of Utrecht University (D. Dissard and A. Dueñas Bohórquez, pers. com.). Only the culture facilities of the BIAF, and of the Woods Hole Oceanographic Institution (USA) concentrate their efforts on the calibration of deep-sea benthic foraminifera such as *Bulimina*. In Chapter 2, we present a literature review of laboratory experiments performed with this genus.

CHAPITRE 2

MORPHOLOGICAL VARIABILITY IN NON-COSTATE BULIMINIDS

CHAPITRE 2

MORPHOLOGICAL VARIABILITY IN NON-COSTATE BULIMINIDS

1. INTRODUCTION

Our work aims to measure the isotopic composition of deep-sea benthic foraminifera that calcified the totality of their test at different temperatures in controlled laboratory conditions. During the last 3 years, we performed numerous culture experiments with assemblages of deep-sea benthic foraminifera from the Bay of Biscay sampled at 450 and 650 m water depth. These preliminary experiments pointed out that non-costate Buliminids were the most successful to live, reproduce and grow in laboratory cultures. Therefore, our experimental study focused on this group. Until now, the classification of the different species of non-costate Buliminids is based on the overall similarity in the morphology and other observable traits (e.g. the composition of the test). There are two common ways to differentiate the species of *Bulimina*: (1) based on the presence/absence of undercuttings and ornamentations (*B. marginata*, *B. aculeata* and *B. elongata*), and (2) based on the general shape of the shell (*B. marginata*, *B. aculeata*, *B. fusiformis*, *B. elegans*, *B. gibba*…). Following the first school, we identified two morphotypes in our foraminiferal assemblages: *B. marginata* f. *marginata* and *B. marginata* f. *aculeata*. However, there is extensive discussion in the literature wether these two morphotypes represent biological species, subspecies, or ecophenotypes of a same genetic species. In the following sections, we propose a concise literature review of the most relevant and recent publications dealing with taxonomy, ecological requirements and paleoceanographical interest of the *B. marginata/B. aculeata* group. This analysis will lead to the pragmatic decision to consider these two groups as two morphotypes of the species *B. marginata*. Finally, we will describe the culture experiments involving these two types of non-costate *Bulimina*.

2. CLASSIFICATION AND DESCRIPTION

The classification and description presented here are mainly based on the supraspecific taxonomical studies of Loeblich and Tappan (1964, 1988). For the part on the specific nomenclature, the reader should realise that our study is not exhaustive.

Family BULIMINIDAE Jones, 1875

The test is trochospiral and triserial (three chambers per whorl) and later may be reduced to biserial. The wall is calcareous. The aperture is a loop in the apertural face with tooth plate that extends backward from the aperture to the previous foramen (Loeblich and Tappan, 1964, 1988).

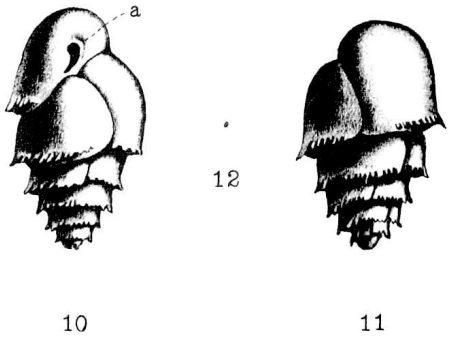
Subfamily BULIMININAE Jones, 1875

The test is triserial and becoming involute. The aperture has a loop-shaped form with a distinct tooth plate (Loeblich and Tappan, 1964).

Genus BULIMINA d'Orbigny, 1826; in Cushman, 1911

The test is triserial in the first ontogenetic stages and may become uniserial in the final part. The wall is calcareous, finely to coarsely perforated, with a radial structure. The aperture extends up from the apertural face, with a free border that may have an elevated rim and a fixed border attached to the internal folded tooth plate, which is attached to the internal chamber wall below the aperture, with a free shank that may be dentate or smooth, flaring or enrolled and subtubular (Loeblich and Tappan, 1964).

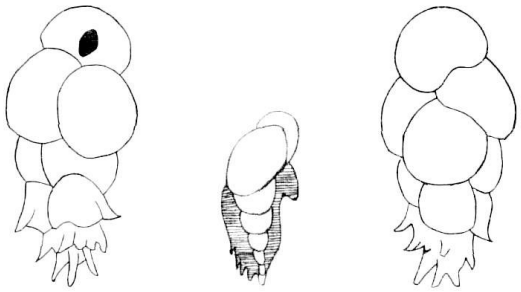
Species ***Bulimina marginata*** d'Orbigny, 1826



1826 *Bulimina marginata* d'Orbigny; Tableau méthodique de la classe des Céphalopodes. Ann. Sci. Nat., Paris, France, série 1, tome 7, p. 269, pl. 12, figs. 10-12.

The test is elongated, ovate to subcylindrical, the chambers are triserially arranged. The chambers are inflated and the sutures are distinct and depressed. The wall is calcareous, finely to coarsely perforate, optically radial with a smooth surface, but the lower margin of chambers may be carinate and spines are frequently developed at the basal border of the chambers and the initial end. According to Jorissen (1988), typical specimens of *B. marginata* are characterized by the presence of broad, sharply angled undercuttings (distinctive angle near to the basal suture of the chamber wall; Verhallen, 1987) at the base of each chamber, and well developed triangular spines aligned at the lower chamber margins. The aperture is loop-shaped, extending up the face from the base of the last chamber, a free border having an elevated rim, and a fixed border continuous with an internal folded tooth plate connecting through the chambers between the apertures.

Species *Bulimina aculeata* d'Orbigny, 1826



4 128 4

1826 *Bulimina aculeata* d'Orbigny; Tableau méthodique de la classe des Céphalopodes. Ann. Sci. Nat., Paris, France, série 1, tome 7, p. 269. Figure in: Parker, Jones and Brady, Ann. Mag. Nat. Hist., London, England, 1871, ser. 4, vol. 8, pl. 11, fig. 128.
1902 *Bulimina aculeata* d'Orbigny; Fornasini, Acc. Sci. Bologna. Mem., Bologna, Italia, 1902, ser. 5, tome 9, p. 153, fig. 4.

The test is large and consists of 4 to 6 whorls and the initial end is subacute. The test is regularly triserial, with distinct chambers, that increase rapidly in size, with a rounded and inflated shape, especially in the last whorl. The sutures are distinct and depressed. The form of *B. aculeata* is very variable with regards to the amount and character of the ornamentation. The wall is often ornamented by spines, either only at the base of the initial part of the test or extending as much halfway up the test. However, some specimens have almost no spines. The spines are usually heavy and short but at times may be quite sharp and long. The remainder of the wall is smooth, very finely perforate and polished. This species is characterized by the absence of undercut margins of the chambers (Jorissen, 1988), and by the fact that, if spines are present, they are found all over the chambers and are not concentrated on the lower part. The aperture is loop-shaped, in a depression of the apertural face, with a raised lip (Cushman and Parker, 1947).

Summarising, the main morphological difference between *B. marginata* and *B. aculeata* is the presence of sharp undercuttings in *B. marginata*, and a concentration of the spines at the base of each chamber. Generally, the length/width ratio of *B. marginata* is low compared to typical *B. aculeata,* which are more elongated. However, a closer inspection of the literature learns

that two different morphotypes are placed in *B. aculeata*. A first morphotype is characterised by the presence of numerous spines all over the chambers at the lower half part of the test (Plate 2.1, Fig. 1). The second morphotype has only a few spines and is more elongated (Plate 2.1, Fig. 2), as described by Jorissen (1988). This type has been identified as a morphotype of *B. marginata* f. *aculeata* by this author.

Also, in the sediment samples from the Bay of Biscay used in our culture experiments, we identified two different morphotypes: *B. marginata* f. *marginata* and *B. marginata* f. *aculeata* (as described by Jorissen, 1988). These two morphotypes have been previously described in this area by Fontanier *et al*. (2002) and Langezaal *et al*. (2006). Plate 2.1 shows typical specimens from the Bay of Biscay (450 and 650 m water depth) of *B. marginata* f. *marginata* (Plate 2.1, Figs. 3-7) and *B. marginata* f. *aculeata* (Plate 2.1, Figs. 8-11) used in our experimental study (Chapters 3, 4 and 5). The distinction between the two morphotypes is the same as the one observed in the central basin of Adriatic Sea at 220 m water depth (Plate 2.1, Figs. 12 and 13; Jorissen, 1988). Intermediate morphotypes are present, but rare. We also present typical specimens of *B. marginata* that we used to establish the *in situ* calibration curve of the $\delta^{18}O$ of foraminiferal shells in function of temperature (Chapter 5) that were sampled in the Rhône prodelta (Plate 2.1, Figs. 14 and 15) and in the Indian Ocean (Plate 2.1, Figs. 16-22). In certain specimens from the Indian Ocean, the spines are not restricted to the base of each chamber (Plate 2.1, Figs. 18 and 20). However, because of their general shape and the presence of undercuts, we consider also these forms as *B. marginata* f. *marginata*.

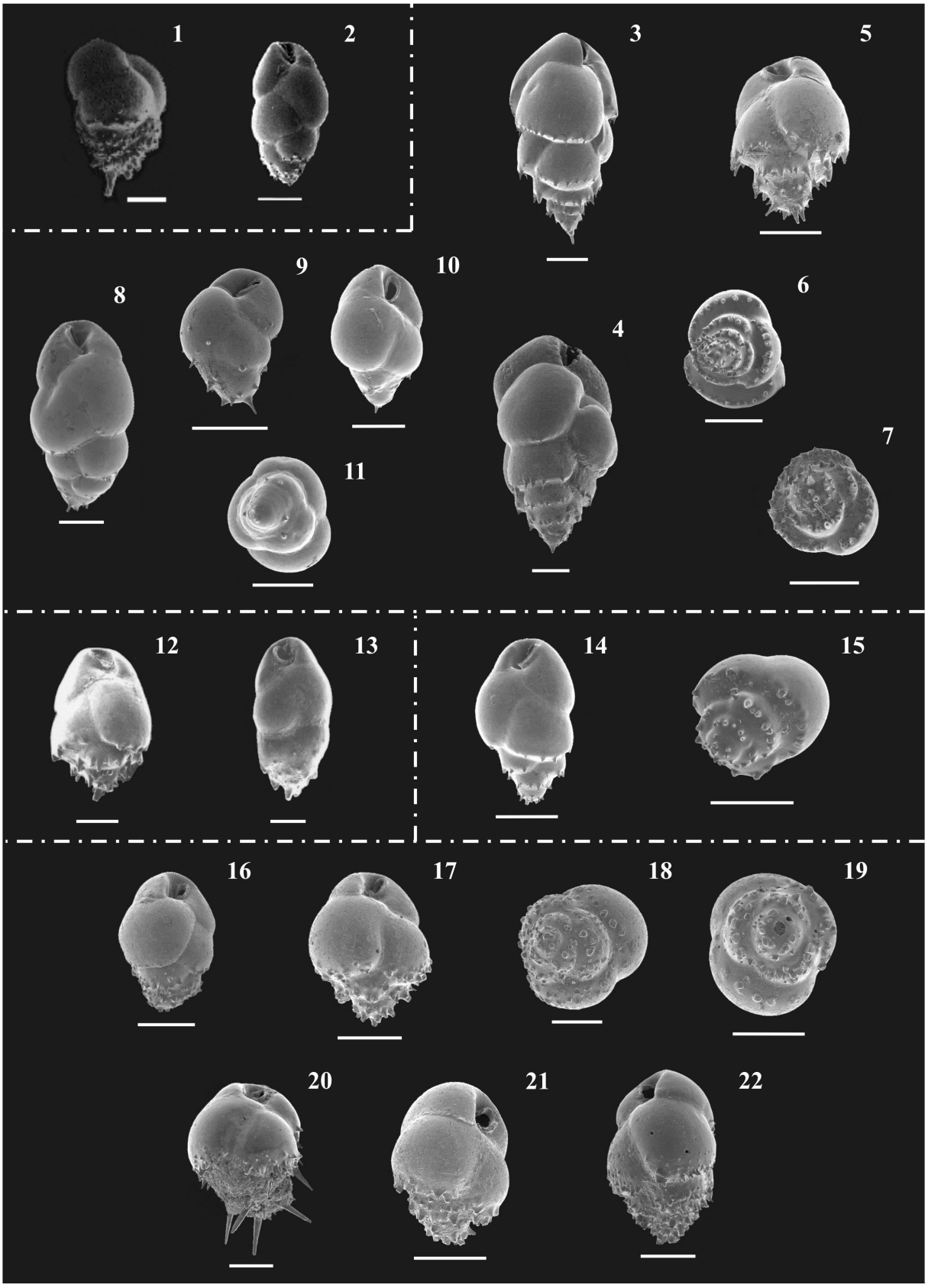

1
2
3
5
8
9
10
6
4
11
7
12
13
14
15
16
17
18
19
20
21
22

Plate 2.1: Fig. 1: B. aculeata *(figure in Mendes* et al.*, 2004); Fig. 2:* B. aculeata *(figure in Abu-Zied* et al.*, 2008); Figs. 3-11: Typical specimens of* B. marginata *f.* marginata *(Figs. 3-7) and* B. marginata *f.* aculeata *(Figs. 8-11) sampled in the Bay of Biscay and used in our culture experiments; Figs. 12-13: Specimens of* B. marginata *f.* marginata *(Figs. 12) and* B. marginata *f.* aculeata *(Figs. 13) observed in the Adriatic Sea at 220 m depth by Jorissen (1988); Figs. 14-15: Typical specimens of* B. marginata *sampled in the Rhône prodelta; Figs. 16-22: Typical specimens of* B. marginata *sampled in the Indian Ocean. Scale bars represent 100 µm.*

3. ECOLOGY AND DISTRIBUTION

3.1. Geographical distribution

Bulimina marginata and *Bulimina aculeata* are both cosmopolitan. Both morphospecies are found in the Pacific Ocean (e.g. Phleger and Soutar, 1973; McCorkle *et al*., 1997; Ohga and Kitazato, 1997; Szarek *et al*., 2006), in the Atlantic Ocean (e.g. Lutze and Coulbourn, 1984; Corliss, 1991; Jorissen *et al*., 1998; Morigi *et al*., 2001; Fontanier *et al*., 2002; Mendes *et al*., 2004; Langezaal *et al*., 2006; Eberwein and Mackensen, 2006; Mojtahid *et al*., 2006, 2008; Koho *et al*., 2007; Eichler *et al*., 2008; Panieri and Sen Gupta, 2008; Pascual *et al*., in press), in the Indian Ocean (e.g. Jannink *et al*., 1998; Murgese and De Deckker, 2007; Smart *et al*., 2007), in the Southern Ocean (Mackensen *et al*., 1995; Igarashi *et al*., 2001), in the Mediterranean Sea (e.g. Jorissen, 1988; De Rijk *et al*., 1999, 2000; Schmiedl *et al*., 2000; Donnici and Barbero, 2002; Ernst *et al*., 2005; Bergin *et al*., 2006; Di Leonardo *et al*., 2007; Mojtahid, 2007; Frezza and Carboni, in press; Pucci *et al*., submitted) and in the North Sea (e.g. de Nooijer *et al*., 2008; Brückner and Mackensen, 2008) (Appendix 2.1). Both *B. marginata* and *B. aculeata* live at a wide range of water depths, from the continental shelf between 10 and 150 m water depth (e.g. Phleger and Soutar, 1973; Lutze and Coulbourn, 1984; Barmawidjaja *et al*., 1992; Fontanier *et al*., 2002; Mendes *et al*., 2004; Mojtahid *et al*., 2006, 2007, 2008; Langezaal *et al*., 2006; Szarek *et al*., 2006; Di Leonardo *et al*., 2007; Eichler *et al*., 2008; Brückner and Mackensen, 2008; Pascual *et al*., in press; Frezza and Carboni, in press) to the continental slope and bathyal environments between 150 and 2200 m water depth (e.g. Lutze and Coulbourn, 1984; Corliss, 1991; Jorissen *et al*., 1998; De Rijk *et al*., 1999, 2000; Mackensen *et al*., 2000; Morigi *et al*., 2001; Eberwein and Mackensen, 2006;

Abu-Zied *et al*., 2008) (Appendix 2.1). These species inhabit also canyon environments, where they are either found along the canyon axis (Schmiedl *et al*., 2000; Hess *et al*., 2005) or in the upper canyon terraces (Koho *et al*., 2007).

In fact, no clear difference in the geographical and bathymetrical distribution range of *B. marginata* and *B. aculeata* is shown in the literature. This is an argument for the distinction of *B. marginata* and *B. aculeata* as two ecophenotypes.
Several studies pointed out that *B. marginata* and *B. aculeata* are both associated with fine grained muddy sediment (Jorissen, 1988; Mackensen *et al*., 1995; Mendes *et al*., 2004). Eberwein and Mackensen (2000) noticed that both species are adapted to sediment with sand content below 15% in the stations studied off Morocco and *B. marginata* is particularly abundant in clayey sediment (Donnici and Barbero, 2002; Frezza and Carboni, in press). Donnici and Barbero (2002) observed that *B. marginata* and other deltaic species living off the Po delta show a negative correlation with substrate diameter.

3.2. Temperature and salinity conditions

According to the literature, *Bulimina marginata* and *B. aculeata* are able to live and calcify in a wide range of temperatures. Indeed, *B. marginata* has been reported living at 3.6°C bottom water temperature in the northwestern Atlantic Ocean at 2200 m water depth (Panieri and Sen Gupta, 2008) and also at approximately 20°C in the South China Sea (Szarek *et al*., 2006), in the Gulf of Izmir (Bergin *et al*., 2006) and in the South Brazilian shelf (Eichler *et al*., 2008). *Bulimina aculeata* is found living at a temperature of 2.3°C at 1450 m water depth in the Pacific Ocean (Ohga and Kitazato, 1997). Specimens of *B. aculeata* were even found in core top and fossil samples from East Antarctica (400-780 m and until 2600 m water depth) where the temperature is around 0.5°C (Mackensen *et al*., 1995; Igarashi *et al*., 2001). This species inhabits environments under a wide range of water temperatures up to 18°C (e.g. the Rhône prodelta; Mojtahid, 2007). *Bulimina* species were found in a salinity range between 32 and 40 in the literature (Appendix 2.1). As for their geographic and bathymetric distributions, no clear distinction in temperature and salinity conditions is established between *B. marginata* and *B. aculeata* according to the literature which tend to prove that they belong to the same species.

3.3. Oxygen and organic matter requirements

In the literature, *Bulimina marginata* has been described as a species living in settings with a high food availability (Lutze and Coulbourn, 1984; van der Zwaan and Jorissen, 1991; Sen Gupta and Machain-Castillo, 1993; Rathburn and Corliss, 1994; Rathburn *et al*., 1996; Debenay and Redois, 1997; Jorissen *et al*., 1998; De Rijk *et al*., 2000; Morigi *et al*., 2001; Donnici and Barbero, 2002; Fontanier *et al*., 2002; Mendes *et al*., 2004; Eberwein and Mackensen, 2006). In the Atlantic Ocean off Cap Blanc, Morigi *et al*. (2001) observed the presence of *B. marginata* in stations with increased organic flux which varies from 6 to 10 $g.m^{-2}.y^{-1}$. It has been suggested that this species shows an opportunistic behaviour. For example, Langezaal *et al*. (2006) observed an important peak of density of *B. marginata* after the spring bloom at the outer shelf of the Bay of Biscay. In the central part of the Sunda Shelf (South China Sea), *B. marginata* was found in an area with a low annual primary production that is seasonally influenced by shallow seasonal upwelling which enhances the organic matter input in winter (Szarek *et al*., 2006). In the study of Donnici and Barbero (2002), a strong positive correlation between the quantity of organic matter and relative density of *B. marginata* was observed in the Adriatic Sea continental shelf. The authors supposed that this species is detritivorous.

Bulimina marginata is also considered as a marker of upwelling areas such as the one off northwestern Africa (Lutze and Coulbourn, 1984; Sen Gupta and Machain-Castillo, 1993; Jorissen *et al*., 1998; Morigi *et al*., 2001; Eberwein and Mackensen, 2006), in the eastern Pacific (Phleger and Soutar, 1984) and in the south-western South China Sea (Szarek *et al*., 2006).

Some authors also considered *B. marginata* as indicative of the influence of fluvial runoff since it is found in high abundance in areas close to the Po river in the Adriatic Sea (van der Zwaan and Jorissen, 1991; Donnici and Barbero, 2002), the Rhône river in the Mediterranean (Mojtahid, 2007), the Ombrone river in the Northern Tyrrhenian Sea (Frezza and Carboni, in press), the Mississippi and Rio Grande rivers in the Gulf of Mexico (van der Zwaan and Jorissen, 1991; Sen Gupta and Machain-Castillo, 1993) and the Plata river off south Brazil (Eichler *et al*., 2008). Its density may increase due to important organic matter supplies by the river (van der Zwaan and Jorissen, 1991).

Also *Bulimina aculeata* is considered as an eutrophic taxa found in areas with high organic content for example in the upwelling region off Morocco around 1000 m water depth, in the

Gulf of Lions at 920 m or on the continental shelf off the Guadiana River (Altenbach *et al*., 1999; Schmiedl *et al*., 2000; Mendes *et al*., 2004; Eberwein and Mackensen, 2006; Murgese *et al*., 2007). Nomaki *et al*. (2005b, 2006) performed *in situ* tracer experiments at 1450 m water depth in the central part of Sagami Bay and observed that *B. aculeata* quickly reacted to algal input and exhibited the highest algal ingestion rate which confirms that they are indicators of eutrophic environments.

However, in order to see if there are different trophic requirements for both *B. marginata* and *B. aculeata,* their simultaneous presence in the same field studies is important. De Rijk *et al*. (2000) studied the distribution of the foraminiferal faunas in the Mediterranean Sea according to the organic matter flux. They observed the same distributional pattern for both morphospecies. Their densities decreased with increasing water depth and also from the more eutrophic eastern part to the oligotrophic western part of the Mediterranean. They concluded that *B. marginata* and *B. aculeata* could be considered as opportunistic taxa as was suggested in several other studies (Lutze and Coulbourn, 1984; van der Zwaan and Jorissen, 1991; Alve and Bernhard, 1995; Donnici and Barbero, 2002; Langezaal *et al*., 2006; Mojtahid *et al*., 2006). In addition, De Rijk *et al*. (2000) noticed that the maximum water depth of *B. marginata* was always deeper than that of *B. aculeata*. They estimated that *B. aculeata* was restricted to sites with a minimum labile organic carbon flux of 3 $g.m^{-2}.y^{-1}$ whereas the lower limit value was 2.5 $g.m^{-2}.y^{-1}$ for *B. marginata*. Eberwein and Mackensen (2006) observed the presence of *B. marginata* and *B. aculeata* in the same area of the Atlantic Ocean off Morocco. They also present the same distributional pattern with higher standing stock associated with highest chlorophyll-a concentration which in turn means high organic matter supply to the sediment.

In many studies, *B. marginata* and *B. aculeata* are considered as good markers of low oxygen conditions (e.g. Phleger and Soutar, 1974; Lutze and Coulbourn, 1984; van der Zwaan and Jorissen, 1991; Sen Gupta and Machain-Castillo, 1993; Ohga and Kitazato, 1997; Bernhard and Sen Gupta, 1999; van der Zwaan *et al*., 1999; Pascual *et al*., in press). Recent specimens of these two species were reported to support periodic episodes of anoxia or hypoxia in the Adriatic Sea (Barmawidjaja *et al*., 1992; Donnici and Barbero, 2002) and in the canyon axis of the Bay of Biscay (Hess *et al*., 2005). Shells were also observed in sapropelitic layers in fossil records of the Mediterranean (Olivieri, 1996; Jorissen, 1999b). Laboratory experiments performed in order to determine the influence of anoxic/hypoxic conditions on foraminiferal

faunas tend to confirm field observations. According to Ernst *et al*. (2005), *B. marginata* was hardly influenced by hypoxia and continue to live in anoxic sediments although they observed higher abundances in oxygenated microcosms. Alve and Bernhard (1995) recorded reproduction events for *B. marginata* in low oxygen conditions. However, they noticed that specimens migrated to the sediment surface and occurred on polychaete tubes when the oxygen condition became lower than 0.2 $ml.l^{-1}$. Bernhard and Alve (1996) also observed, by ATP extraction analyses, that *B. marginata* specimens incubated in nitrogen-flushed experimental chambers exhibited lower metabolic activity than specimens from control chambers. Therefore, they conclude that low oxygen concentration seemed to have a negative influence on this species.

The low oxygen conditions of the environment where *Bulimina* spp. are found are generally associated with high organic contents (e.g. sapropels, Oxygen Minimum Zone and upwelling areas) so that the influence of each individual parameter is difficult to determine. Rathburn and Corliss (1994) and Rathburn *et al*. (1996) noticed that species indicative of low oxygen conditions such as *Bulimina*, *Uvigerina* and *Bolivina* were abundant in shallow water sites (500-1000 m water depth) of the Sulu Sea and South China Sea but do not occur in significant numbers in deeper water (below 1000 m) where bottom-water oxygen levels are similar but organic carbon values are lower. They concluded that the oxygen concentration does not determine the density of these "low-oxygen species". *Bulimina marginata* and *B. aculeata* can therefore be considered as tolerant to stress which may prefer however well oxygenated conditions (Alve and Bernhard, 1995; Donnici and Barbero, 2002; Martins and Gomes, 2004; Ernst *et al*., 2005; Mojtahid *et al*., 2006).

3.4. Microhabitat

The microhabitat occupied by *Bulimina* species is not well defined in the literature. Some authors classify *B. marginata* and *B. aculeata* as shallow infaunal taxa, i.e. found within the two first centimetres of sediment (e.g. Corliss, 1991; van der Zwaan and Jorissen, 1991; Rathburn and Corliss, 1994; Alve and Bernhard, 1995; Rathburn *et al*., 1996; McCorkle *et al*., 1997; van der Zwaan *et al*., 1999; Shönfeld, 2001; Martins and Gomes, 2004; Mojtahid, 2007). In several other studies, these species are considered as intermediate to deep infaunal taxa because they present a peak of density in deeper sediment or living specimens are found in the anoxic sediment (e.g. Ohga and Kitazato, 1997; Schmiedl *et al*., 2000; Mackensen *et*

al., 2000; Morigi *et al*., 2001; Fontanier *et al*., 2002). We can see that the distribution of *B. marginata* and *B. aculeata* in the sediment is not clearly delineated. However, if we look in more detail, there is a distinctive pattern that appears in different studies. In the review of Jorissen (1999a) on the microhabitat of benthic foraminifera, the author reported that *Bulimina* species have the particularity to show a profile with a surface maximum and one or several maxima deeper in the sediment (see Figs. 10.6d and 10.7f in Jorissen, 1999a). This pattern was observed for *B. marginata* in the Atlantic Sea off Cap Blanc at 1200 m water depth (Jorissen *et al*., 1998); and for *B. aculeata* in the Pacific (facing the Central Japan) at 1500-1900 m water depth (Kitazato, 1989) and in the Atlantic (off Bouvet Island) at 940 m water depth (Mackensen *et al*., 2000). Fontanier *et al*. (2002) also observed a bimodal distribution of *B. marginata* and *B. aculeata* in the Bay of Biscay at 140 and 550 m water depth, with living specimens found until 9 cm sediment depth. Also in the study of Pucci *et al*. (submitted) testing the vertical distribution of foraminifera according to different experimental conditions, *B. marginata* exhibited a similar pattern in different cores under oxic and hypoxic conditions. However, McCorkle *et al*. (1997) observed no consistent distribution in the North Atlantic Ocean (North Carolina, USA), where *B. aculeata* shows a maximum density in the sediment surface in some cores, whereas in a core sampled at 825 m depth, specimens are abundant throughout the 10 cm of sediment.

From all these observations, we can conclude that this taxon may find suitable environmental conditions at the sediment surface as well as deeper in the sediment. This suggests that *B. marginata* and *B. aculeata* may be able to feed on labile as well as more refractory food, in oxic as well as in anoxic sediments (see § 3.3). Several authors also suggested that *Bulimina* taxa are probably able to migrate rapidly in the sediment which could explain the wide range of their vertical distribution (Barmawidjaja *et al*., 1992; Schmiedl *et al*., 2000; Mackensen *et al*., 2000; Ernst *et al*., 2005).

4. PALEOCEANOGRAPHIC INTEREST

In paleoceanographic reconstructions based on foraminiferal assemblages, the summed relative abundances of the species *Bulimina* spp., *Bolivina* spp., *Uvigerina* spp. and sometimes *Melonis* spp. have been used as indicators of the organic matter flux to the sea floor (e.g. Kawagata *et al*., 2005; Smart *et al*., 2007). Indeed, all these “high productivity” taxa have been observed in areas of high and continuous food supply to the sediment in the

present oceans. Jian *et al*. (1999) estimated, in a study of benthic foraminiferal faunas from the South China Sea (SCS), a threshold of 3.5 $gC.m^{-2}.y^{-1}$ of organic carbon flux above which *B. aculeata* and *U. peregrina* become dominating species. In this study, the high relative abundances and Accumulation Rates (AR) of these two species correspond to an increased organic carbon flux identified in the southern SCS during the Last Glacial Maximum and in the northern SCS during the early Holocene. In the eastern Indian Ocean, Murgese *et al*. (2007) also used the AR of *B. aculeata*, as well as *Epistominella exigua* and *Uvigerina proboscidea*, with maximal AR's as indicators of episodes of increased organic matter supply to the sea floor over the last 30 kyrs. In the subantarctic area, *B. aculeata* and *B. marginata* are used as markers of the Circumpolar Deep Water (CDW) (Igarashi *et al*., 2001; Hayward *et al*., 2007). Indeed, the presence of the warm, nutrient-rich and $CaCO_3$-saturated CDW promote the calcification of calcareous species of foraminifera and more specially species as *B. marginata* and *B. aculeata* which respond to high organic matter input by increased densities.

The stable isotopic composition ($\delta^{18}O$, $\delta^{13}C$) of benthic foraminifera is also used as proxies to reconstruct paleoceanographic characteristics (e.g. paleotemperature, paleoproductivity). The species used for these reconstructions are usually ubiquists with a narrow and well defined microhabitat. Epifaunal species (e.g. *Cibicidoides*) are supposed to record the geochemical composition of the bottom water, whereas shallow (e.g. *Uvigerina*), intermediate (e.g. *Melonis*) and deep infaunal species (e.g. *Globobulimina*) record pore water chemistry from different sediment depths. Although *B. marginata* and *B. aculeata* are cosmopolitan and are present in a wide range of water depth and bottom water temperatures, only few studies analysed their isotopic composition (Rathburn *et al*., 1996; McCorkle *et al*., 1997; Mackensen *et al*., 2000; Mackensen and Licari, 2004; Eberwein and Mackensen, 2006; Abu-Zied *et al*., 2008; Brückner and Mackensen, 2008). Their ability to migrate in the sediment and to occupy different microhabitats may be a reason explaining the lack of paleoceanographic interest of these species. However, the data obtained until now, presented in the following paragraph, are encouraging to continue the investigation of the isotopic composition of *Bulimina*.

McCorkle *et al*. (1997) performed isotopic measurements on living (Rose Bengal stained) specimens of *B. aculeata* sampled in the north-western Atlantic Ocean off North Carolina. They observed that *B. aculeata* is calcifying close to equilibrium with the oxygen isotopic composition of bottom seawater with an average offset ($\Delta\delta^{18}O$; $\delta^{18}O_{foram}$-$\delta^{18}O_{seawater}$) of

-0.2‰ (McCorkle *et al*., 1997). On the opposite, they observed a larger $\Delta\delta^{18}O$ for species such as *Cibicidoides, Melonis* and *Elphidium* ($\Delta\delta^{18}O$ values up to 1‰). Therefore, it appears that the offset due to vital effect is higher for the latter species than for *B. aculeata* where it is almost negligible. No difference in the $\delta^{18}O$ composition of specimens sampled at different sediment depths was observed. This is consistent with the fact that bottom water and pore water oxygen isotopic compositions are equal since there is no change in temperature or salinity within the sediment.

The $\delta^{13}C$ composition of *B. aculeata* specimens reveals a negative offset compared to the bottom water isotopic composition (0.6 to 1.4‰ lower) at the sites studied by McCorkle *et al*. (1997). These values are typical of shallow infaunal species since the $\delta^{13}C$ values are more depleted than for epifaunal species but less depleted than for deep infaunal species (see Fig. 16 in McCorkle *et al*., 1997). The authors considered that this offset is mainly due to the rapid decrease in the $\delta^{13}C$ composition of pore water with increasing depth in sediment because of the degradation of the organic matter. In this case, we would expect the $\delta^{13}C$ composition of one given species to decrease with increasing depth. However, no variation in the $\delta^{13}C$ values was observed for *B. aculeata* or other species according to sediment depth. The same pattern was observed for *B. aculeata* sampled in the southern Atlantic Ocean off Bouvet Island (Mackensen *et al*., 2000; Mackensen and Licari, 2004) and for *B. marginata* sampled in Cap Blanc (Griveaud, 2007). One possible explanation could be that specimens migrate during their lifespan within the sediment but calcify within a narrow geochemically homogeneous zone within the full depth range of sediment (McCorkle *et al*., 1997). Since *Bulimina* tends to show an opportunistic behaviour, this hypothesis could be supported by a rapid growth event initiated by a high seasonal input of organic matter. In the case of *B. aculeata* and *B. marginata*, it is clear from the literature that these species can be found throughout the sediment column and do not exhibit a particular microhabitat. For this reason, another explanation could be that specimens are able to migrate in the sediment and calcify different chambers at different depths within the sediment. The carbon isotopic composition of this species would be the result of an average isotopic pore water signal and living specimens caught at different sediment depths would exhibit the same $\delta^{13}C$ composition (Mackensen *et al*., 2000). Nevertheless, the recorded signal depends on surface water productivity and in turn, on the organic carbon exported to the sediment. Mackensen *et al*. (2000) recorded the same $\Delta\delta^{13}C$ of about 0.6±0.1‰ for specimens of *B. aculeata* sampled at all stations off Bouvet Island except for one (PS2094). They interpret this higher offset at this

station (-1.5±0.23‰) as indicative of a higher local organic matter flux. According to Mackensen *et al*. (2000), differences in average annual primary production of around 30 $gC.m^{-2}.y^{-1}$ would result in approximately 1‰ difference in $\delta^{13}C$ values of *B. aculeata* tests. Therefore, the carbon isotopic composition of *B. aculeata* is of interest for paleoceanographic studies, as it would give information on the average organic matter flux at one particular site and one particular time.

The study of Mackensen *et al*. (1993) compared the isotopic composition of living specimens, (full of cytoplasm) and dead specimens (empty shells) sampled at different water depths and different trophic conditions. The authors observed no systematic difference, and concluded that the signal is kept in the calcite of empty tests without further significant modification during early sedimentation history. Therefore, the conclusions achieved in the previously cited studies based on isotopic measurements performed on living (Rose Bengal stained) foraminifera should be relevant for the study of dead or fossil *Bulimina*.

Finally, we can see that in the literature, more isotopic data are available for *B. aculeata* than for *B. marginata*. Since these two species have the same ecological requirements, we can expect that the same isotopic fractionation would be observed for both taxa. It is however important to keep in mind a possible interspecific difference in isotopic composition. Rathburn *et al*. (1996) reported a difference in the $\delta^{13}C$ composition of *B. marginata* (-0.58‰) and *B. mexicana* (-1.08‰) sampled from the same interval of a same core in the Sulu Sea and concluded that there may be intrageneric differences among *Bulimina* species.

5. CULTURE EXPERIMENTS WITH BULIMINA

Bulimina species appear to be well adapted to laboratory culture experiments of benthic foraminifera. Culture experiments have been used for ecological purposes (e.g. Alve and Bernhard, 1995; Hemleben and Kitazato, 1995; Bernhard and Alve, 1996; Gross, 2000; Bernhard *et al*., 2004; Ernst *et al*., 2004, 2005; Nomaki *et al*., 2005a) or in order to better calibrate these proxies for paleoceanographic studies (e.g. Wilson-Finelli *et al*., 1998; Havach *et al*., 2001; McCorkle *et al*., 2004; Hintz *et al*. 2004, 2006a, 2006b).

The ecological experiments mainly aimed to determine the impact of different trophic and oxygen conditions on shallow (Alve and Bernhard, 1995; Bernhard and Alve, 1996; Ernst *et al*., 2005) and deep benthic foraminifera assemblages (Ernst *et al*., 2004; Nomaki *et al*.,

2005a), including *B. marginata* or *B. aculeata*. Ernst *et al*. (2004, 2005) performed experiments in mesocosm with foraminiferal assemblages from the Bay of Biscay (550 m water depth) and from the Adriatic Sea (32 m water depth). The response of *B. marginata* specimens was slightly different in the two studies. In one case, the low oxygen conditions had no clear impact on the distribution of the specimens in the sediment but the high organic matter input in one of the mesocosm induced an abundance increase at the sediment surface (Ernst *et al*., 2005). From this study, oxygen concentration appears not to be a determinant parameter for the distribution of *B. marginata*. However, in the other study of Ernst *et al*. (2004), cultured *B. marginata* exhibited a shallower microhabitat under dysoxic conditions than under oxygenated conditions. Also, Alve and Bernhard (1995) observed the migration of the specimens to the sediment water interface or to polychaete tubes in order to stay in oxygenated conditions. Experiments realised *in situ* with labelled ^{13}C algae in Sagami Bay at 1450 m depth aimed to study the uptake of organic carbon by the different species of the living assemblage (Nomaki *et al*., 2005b) and to differentiate selective feeding on different type of food (Nomaki *et al*., 2006). The *in situ* tracer experiments demonstrated that *B. aculeata* quickly reacted to algal deposition with the highest ingestion rate of all tested species. Moreover, the authors demonstrated that this species was able to feed on the unicellular marine algae *Dunaliela tertiolecta* but also to ingest bacteria. To summarise, we can deduce from these experiments that *B. marginata* and *B. aculeata* are mainly dependant on food supply. They prefer to live in oxic conditions but they are able to survive stressed conditions like to changes in redox conditions. These culture observations confirm the observations made in the field and explain their capacity to inhabit surface sediment as well as deeper sediment layers. The migration speed of *B. marginata* specimens was estimated in the studies of Hemleben and Kitazato (1995) and of Gross (2000) at around 1 and 2.7 $\mu m.min^{-1}$. Comparatively to the other species tested, Gross (2000) classified this species in the "fast" migration speed class. Therefore the idea proposed by Mackensen *et al*. (2000) according to which the high migration of *Bulimina* species in the sediment would result in an averaged isotopic signal seems to be plausible. However, these experimental data must be taken with care since they were obtained in conditions different from the natural environment.

Since *Bulimina* specimens succeeded to reproduce in experimental setups, several experimental studies performed geochemical measurements on *Bulimina* shells that calcified in controlled conditions. Havach *et al*. (2001) and Wilson-Finelli *et al*. (1998) set up experiments in sediment microcosms to determine, respectively, the trace element partition

coefficient (of Barium and Cadmium) and stable isotopic composition of foraminiferal calcite cultured under stable conditions. Sediment microcosms aim to mimic as far as possible the natural environment, in order to study the influence of the microhabitat on the geochemical composition of the shell of different species. In both studies, reproduction of *B. marginata* occurred and measurements were performed on juvenile specimens that calcified the totality of their test in the controlled conditions. Havach *et al*. (2001) obtained values of Ba/Ca and Cd/Ca partition coefficients close to those obtained with field samples. The oxygen and carbon isotopic data obtained from specimens of *B. marginata* calcified in replicate microcosms displayed similar values (Wilson-Finelli *et al*., 1998). The foraminiferal $\delta^{18}O$ values were consistently enriched by 0.07 to 0.50‰ compared to the seawater isotopic composition. However, the carbon isotopic composition of the seawater in the microcosms was not entirely kept stable so that the interpretations are open to discussion.

Another experimental set up consisted in culturing deep-sea foraminifera without natural sediment in order to avoid any microhabitat effect (Hintz *et al*. 2004). McCorkle *et al*. (2004) and Hintz *et al*. (2006a, 2006b) performed cultures in such an experimental system with an assemblage of foraminifera off South Carolina to measure stable isotopes and trace metal elements. *Bulimina aculeata* and *Rosalina vilardebouana* were the only two species that succeeded to reproduce within the 4 to 9 months duration of the experiments. Cultured specimens of *B. aculeata* calcified close to equilibrium with the $\delta^{18}O$ composition of seawater. The recorded $\Delta\delta^{18}O$ values, between -0.3 and 0.1‰, were on average slightly lower than the $\Delta\delta^{18}O$ values of field specimens (McCorkle *et al*., 2004). The $\delta^{13}C$ values were lower than the carbon isotopic composition of the seawater and close to the field data. However, the authors explained that the depletion in both field and culture $\delta^{13}C$ values is due to different effects; the microhabitat effect for field data (McCorkle *et al*., 1997) and the carbonate ion effect (Spero *et al*., 1997) for cultured specimens, because the experimental seawater had particularly high alkalinity and pH values. They also measured an ontogenetic effect on both *B. aculeata* and *R. vilardebouana* and more depleted isotopic composition for *R. vilardebouana* compared to *B. aculeata* calcified in the same conditions (McCorkle *et al*., 2004). Both observations indicate that species-dependent vital effects influence foraminiferal isotopic composition.

The results of the experiments performed on *B. marginata* and *B. aculeata* have led to a meaningful series of data of stable isotope and trace metal elements. Therefore, experiments with these species are very promising. The next step should be to carry out experiments in

different conditions, with only one parameter changing in order to determine the influence of this particular parameter on the geochemical composition of *Bulimina* species shell.

6. SYNTHESIS AND CONCLUSION

On the basis of the existing literature, it is difficult to determine if our *marginata* and *aculeata* types should be considered as two different biological species, subspecies, or if they are different morphotypes of a single polymorphic species. This subject has been debated for many years.

Originally, according to the taxonomical description of d'Orbigny (1826), *B. marginata* and *B. aculeata* have been regarded as two distinct species on the bases of chamber angularity, arrangement and length of the spines, and test dimension. In their studies of the morphologic variation of these two species in the Gulf of Maine area, both Collins (1989) and Burgess and Schnitker (1990) concluded that *B. marginata* could be distinguished morphologically from *B. aculeata*. On the other hand, Hoeglund (1947) made precise morphological analyses of both species and found that morphological characteristics changed gradually from one species to the other. He concluded that they were not separate species but morphological variants of the same species. Jorissen (1988) supported the same hypothesis in his study of *Bulimina* morphotypes in the Adriatic Sea. The author distinguished three morphotypes within one single species *Bulimina marginata*: *B. marginata* f. *marginata, B. marginata* f. *aculeata* and *B. marginata* f. denudata. Between 30 and 50 m depth, these morphotypes intergrade, whereas they become well separated in deeper water. The same nomenclature was employed by Hayward *et al*. (1999) in their review on shallow-water benthic foraminifera from New Zealand. Moreover, in the study of Kitazato *et al*. (2003), which is the only work until now were DNA analysis were performed on 8 morphotypes of the genus *Bulimina*, *B. marginata* and *B. aculeata* belong to the same cluster in the phylogenetic tree. The authors measured a low degree of divergence within the group *B. aculeata*, *B. marginata*, *B. elongata* and *B. pagoda*. From this study, using other criteria than morphological analyses to determine if these two "species" are actually different species, the authors concluded that they are very close genetically.

The most relevant and recent ecological studies dealing with *B. marginata* or *B. aculeata* tend to prove that they have the same ecological requirements. They are both ubiquist. The wide

range of temperatures (around 3 to 20°C) and salinities (around 32 to 40‰) in which they are living is similar (§ 3.2; Appendix 2.1) even if *aculeata* types are found at lower temperature around the Antarctic (Mackensen *et al*., 1995; Igarashi *et al*., 2001). They are considered as opportunistic taxa by several authors and they are used as indicators of high productivity area (see § 3.3) and high organic matter flux periods in paleoenvironmental studies (e.g. Kawagata *et al*., 2005; Smart *et al*., 2007; Murgese *et al*., 2007). They are also able to support dysoxic to anoxic conditions (see § 3.3). Both morphotypes can be classified as "preferentially" infaunal taxa that present the same vertical distribution in the sediment (see § 3.4). In particular, they can display a bimodal distribution with a maximum density in the upper centimetre of sediment and another peak of high abundance deeper in the sediment (e.g. Jorissen, 1999a).
From the literature, we can therefore conclude that *B. marginata* and *B. aculeata* present similar ecological characteristics. Consequently, also from an ecological point of view, *B. marginata* and *B. aculeata* can be considered as two ecophenotypes of a single biological species, *B. marginata*.

However, we are aware that they are also elements to argue that they could be two distinct species. Except the morphological differences, De Rijk *et al*. (2000) suggested that the minimum labile organic matter flux required for the development of *B. marginata* would be slightly lower than for *B. aculeata*. The results of our geochemical experiments (CSI, CSII and PD) presented in Chapter 4 performed with *marginata* and *aculeata* morphotypes give us new arguments in the favour of two genetically separated different taxa. If we look at the morphotype of the juveniles produced in our experiments, *marginata* morphotype (Plate 2.2, Figs. 1-11) and/or *aculeata* morphotype (Plate 2.2, Figs. 12-15), according to the morphotype of the adults introduced in these experiments, we observed that (Table 2.1):

(1) Juveniles "*aculeata* type" were only found when adults *aculeata* type were introduced (PD-7.9, PD-10.2, PD-12.7, PD-14.7 and PD-13.0). This suggests that adults *marginata* type exclusively produce juveniles *marginata* type, and the same for *aculeata* type;

(2) Juveniles *aculeata* type were only produced when the experimental temperature was higher than 12°C (CSI-12.7, CSI-14.7, CSII17.2, CSII-19.3 and PD-15.7) although the same numbers of adults *aculeata* type were introduced in all the experiments of a

given system (CSI or CSII). Therefore, it could be possible that *aculeata* types can reproduce in a narrower temperature range than *marginata* types;

(3) Juveniles of both morphotypes, *marginata* and *aculeata*, were produced within one experimental bottle (CSI-12.7, bottle B; CSI-14.7, bottle A; CSII-17.2 and CSII-19.3, bottles A). If we consider that *marginata* and *aculeata* are two ecophenotypes, this involves that we should obtain a single morphotype rather than two different ones. Yet, in one experiment bottle, the conditions are homogeneous (same salinity, temperature, type of food…) therefore it would be expected, in a given experiment bottle, to find juveniles of only one morphotype.

Expériences	CSI								CSII						PD					
	CSI-7.9		CSI-10.1		CSI-12.7		CSI-14.7		CSII-4.1	CSII-6.0	CSII-9.3	CSII-11.6	CSII-17.2	CSII-19.3	PD-7.9	PD-10.2	PD-12.7	PD-14.7	PD-15.7	PD-13.0
Température (°C)	7.9		10.1		12.7		14.7		4.1	6.0	9.3	11.6	17.2	19.3	7.9	10.2	12.7	14.7	15.7	13.0
Bouteille expérience	A	B	A	B	A	B	A	B	A	A	A	A	A	A						
Introduction d'adultes du morphotype ***marginata***	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Introduction d'adultes du morphotype ***aculeata***	x	x	x	x	x	x	x	x	x	x	x	x	x	x					x	
Obtention de juvéniles du morphotype ***marginata***	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Obtention de juvéniles du morphotype ***aculeata***						x	x						x	x					x	

Table 2.1: Summary of the morphotypes of adult specimens of Bulimina marginata *(senso lato) introduced in the geochemical experiments CSI, CSII and PD, and the morphotypes of the juveniles produced in these experiments.*

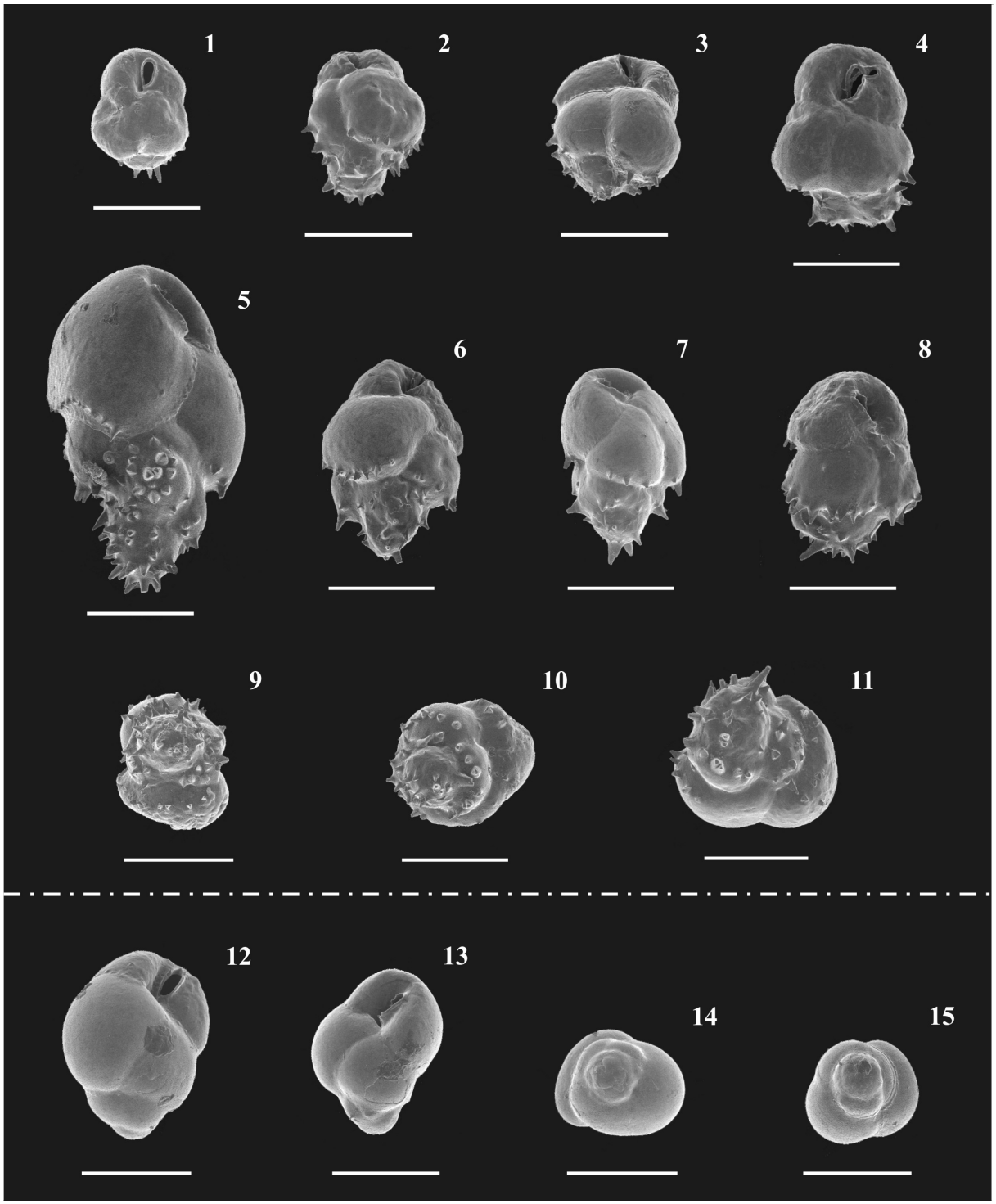


Plate 2.2: Figs. 1-11: Juveniles of B. marginata *f.* marginata *(different sizes) produced and grown in our culture experiments under controlled conditions; Figs. 12-15: Juveniles of* B. marginata *f.* aculeata *produced and grown in our culture experiments under controlled conditions. Scale bars represent 100 µm.*

According to these new observations, particularly the argument (1), there is a possibility that *B. marginata* and *B. aculeata* are actually two distinct species, or two subspecies in the course of speciation. However, this conclusion would be based on punctual observations which are in any case insufficient to rule on this controversial subject. Moreover, in our growth experiments where different conditions of temperature and food were tested on specimens of *marginata* and *aculeata* morphotypes (Chapter 3), we observed no specific difference in the growth rate of the shells between the two morphotypes.

Finally, the present evidences are insufficient to decide on the classification of *B. marginata* and *B. aculeata*. Further genetic studies on specimens of these two groups are needed.
For pragmatic reasons, we decided to consider *B. marginata* and *B. aculeata* as two morphotypes of a single biological species *Bulimina marginata*. Knowing that the *marginata* morphotype was largely dominant in all our experiments in laboratory, this choice allowed to lighten considerably the text of the manuscript. Actually, our experiments were either performed with only *marginata* morphotypes or with a large majority of this morphotype (more than 80% of *marginata* morphotype except in one experiment). However, we always precise in the text to which morphotypes belong the specimens used in our experiments.

According to previous culture experiments, *Bulimina marginata* (*marginata* and *aculeata* morphotypes) specimens sampled in deep sea environments are able to reproduce and grow in laboratory conditions (Wilson-Finelli *et al*. 1998; Havach *et al*. 2001; Bernhard *et al*., 2004; McCorkle *et al*., 2004; Hintz *et al*., 2004). However, reproduction events did not occurred in a systematic way and the factor stimulating reproduction was not identified. Therefore their experiments lasted between 4 months to 3 years to obtain sufficient calcite formed in controlled conditions. In the next chapter of this manuscript (Chapter 3), we present the results of laboratory experiments designed to find out the optimal conditions of food and temperature to obtain reproduction and growth of *B. marginata*. The aim was to be able to stimulate reproduction and growth in a limited time to optimize the duration of the experiments. Indeed, these preliminary experiments allowed us to perform laboratory cultures of *B. marginata* under controlled conditions to analyse stable isotopes as done by Wilson-Finelli *et al*. (1998) and McCorkle *et al*. (2004). To go into more details, the bonus of our study is that we carried out experiments at different temperature conditions in order to establish the influence of this particular parameter on the $\delta^{18}O$ composition of *B. marginata* (Chapter 4 and 5). In Chapter 5, the isotopic data obtained for *B. marginata* f. *marginata* and

B. marginata f. *aculeata* were treated separately. Indeed, although the similarity in their ecological requirements is pretty clear, the few isotopic data available in the literature are for the moment not sufficient to ensure that these two morphospecies present the same fractionation patterns.

Appendix 2.1 *: Environmental parameters (location, bathymetry, temperature, salinity, microhabitat and sediment type) characterizing* Bulimina marginata *and* Bulimina aculeata *in the most relevant and recent publications.*

Authors	Year	Species	Study area	Localisation	Occurrence Water depth	Bottom Water Temperature	Bottom Water Salinity	Microhabitat	Sediment type
Phleger and Soutar	1973	*Bulimina* spp.	Upweling eastern Pacific, Baja California		75-100m	6-11°C			
Lutze and Coulbourn	1984	*B. marginata*	Continental margin of northwest Africa	Between the Straits of Gibraltar and Dakar, Senegal					
Jorissen	1988	*marginata* and *aculeata* morphotypes	Adriatic sea	45°-42°N and 12°-16°E	30-300m	10-15°C	37-38	Infaunal	Clay (*marg.*); Clay + sand (*acul.*)
Corliss	1991	*Bulimina* spp.	Northwest Atlantic Ocean	41°40-42°35N and 64°11-69°53W	1075m to 1575m for *B. marg.*			Shallow infaunal (0-2cm interval)	
Corliss	1991	*B. marginata*	Nova Scotian continental margin	43°N/60°W	200m	6-12°C		Shallow infaunal	
Van der Zwaan and Jorissen	1991	*B. marginata*	Adriatic sea and Gulf of Mexico					Epifaunal for *Bul.* f. *marg.*; predominantly infaunal for *Bul.* f. *acul.*	
Barmawidjaja et al.	1992	*B. marginata*	Northern Adriatic Sea	44°45.4'N/12°45'E	32m	9.5-16.5°C	37-38	"Potentially infaunal"	
Jorissen et al.	1992	*B. marginata*	Northern Adriatic Sea	44°09'-45°21'N and 12°34'-13°30'E	28-51m	14.3-17.8°C	36.8-38.1	Potentially infaunal	outer edge of the clay-belt
Rathburn and Corliss	1994	*B. marginata*	Sulu Sea	8°2.9'N/118°22.4'E	510m	11.06°C	34.46	Peak at 0-1cm and sp. found between 0 and 7cm	
Alve and Bernhard	1995	*B. marginata*	Inner Oslo Fjord, Norway	59°44.28'N/10°31.75'E	71m	7-10°C (*in situ*)	32.5-33.8 (*in situ*)	Peak at 0.5cm and specimens found until 2cm (initial conditions)-epifaunal	
Mackensen et al.	1995	*B. acuelata*	South Atlantic Ocean	Atlantic sector of circumpolar ocean	until 2600m	until 0.5°C			Fine-grained sediment
Bernhard and Alve	1996	*B. marginata*	Drammensfjord, Norway						

Appendix 2.1 *(Continued)*

Authors	Year	Species	Study area	Localisation	Occurrence Water depth	Bottom Water Temperature	Bottom Water Salinity	Microhabitat	Sediment type
Rathburn et al.	1996	*B. marginata*	Sulu Sea (SS) and South China Sea (SCS)	8°2.9'N/118°22.4'E and 10°57.3'N/118°27.5'E	510m (SS) and 1095m (SCS)	11.06°C	34.46	Shallow infaunal (0-2cm)	
Debenay and Redois	1997	*B. marginata*	Off northern Senegal	14°50'-16°20'N	Shelf and uppermost slope down to 180m				
McCorkle et al.	1997	*B. aculeata*	Off North Carolina, Pacific Ocean	35°15'-36°13'N and 74°32'-74°50'W	577, 720, 825, 1477m	4.32-4.73°C		Shallow infaunal (max. in 0.5-1cm, stained sp. until 6cm); at 820m, sp. throughout the 10cm	
Ohga and Kitazato	1997	*B. aculeata*	Central Sagami Bay	35°00'N/139°22.5'E	1450m	2.3°C	35		
Jannink et al.	1998	*B. aculeata*	Continental margin South of Karachi, Pakistan	22°11'-24°13'N and 65°27'-66°03'E	1250-1500m	5.2-7.3°C			
Jorissen et al.	1998	*B. marginata*	Upwelling of Cap Blanc, Northwest Africa	21°25'N/18°04.2'W and 21°28.8'N/17°57.2'W	1525 and 1195m	5.8°C (1200m) and 4.7°C (1500m)		Deep infaunal (ALD=5.87cm (1525m) and 3.53cm (1195m), surficial+downcore max.)	
De rijk et al.	1999	*B.marginata* / *B. aculeata*	Mediterranean Sea		500-1000m for *B. marg.;* 0-500m for *B. acul.*				Upper slope
De Rijk et al.	2000	*B.marginata* / *B. aculeata* (recently dead)	Mediterranean Sea	40°N/20°E and 36°N/2°W	700-2000m for *B. marg.;* 500-1500m for *B. acul.*	13-14.6°C			
Mackensen et al.	2000	*B. aculeata*	South Atlantic Ocean close to Bouvet Island	48-55°S	500 to 1600m			surface maximum (0-1cm) and found consistently down to 5cm in sediment	
Schmiedl et al.	2000	*B. aculeata*	Gulf of Lions, Mediterranean Sea	Canyon axis 42°27.60N/38°29.80E	920m	12.9°C	38.45	Preferentially deep infaunal (ALD>3cm)	

Appendix 2.1 *(Continued)*

Authors	Year	Species	Study area	Localisation	Occurrence Water depth	Bottom Water Temperature	Bottom Water Salinity	Microhabitat	Sediment type
Igarashi et al.	2001	*B. aculeata* (core top + fossils)	Eastern part of Lützom-Holm Bay, East Antarctica	69°S/39°E	400 to 780m	around 0.5°C			
Morigi et al.	2001	*B. marginata*	Eastern Atlantic Ocean bordering Northwest Africa	19-27°N and 18-14°W	750-1550m			Intermediate to deep infauna	
Shönfeld	2001	*B. marginata*	Gulf of Cadiz and southwestern Iberian Margin	36-38°N and 10-7°W				Shallow infaunal	
Donnici and Barbero	2002	*B. marginata*	Off the Po Delta, northern Adriatic Sea	45°10'-44°20'N and 12°25'-13°15'E	around 10-40m				clayey sediment
Fontanier et al.	2002	*B.marginata* / *B. aculeata*	Bay of Biscay, northern Atlantic Ocean	43°41'93N/1°34'10W	140m (both species); 550m for *B. marg.*)	11.9°C	35.6	Deep infaunal (station D, ALD=4.2 & 4.9cm for *B. marg.* & *B. acul.*, resp.)	
Mendes et al.	2004	*B.marginata* / *B. aculeata*	Continental shelf off Guadiana River	37°N/7.4°W	> 95m for *B. marg.* and 30-100m for *B. acul.*	15°C			fine-grained mud deposits
Ernst et al.	2005	*B. marginata*	Off the Italian coast (continental shelf), Adriatic Sea		32m	12-13°C (*in situ*) and 16°C (in exp.)	37.4-38.3 (*in situ*) and 37.5 (in exp.)	No clear max. density, found throughout the sediment column	
Hess et al.	2005	*B. marginata*	Cap Breton Canyon, Bay of Biscay	43°38'N/1°43'W	650m				
Bergin et al.	2006	*B. marginata*	Gulf of Izmir, Turkey	38°50N/26°70E	15-66m	19.15±0.01°C and 17.72± 0.02°C	32.11–39.66 and 36.97-39.10		
Eberwein and Mackensen	2006	*B.marginata* / *B. aculeata*	Upwelling region off Morocco between Cape Ghir (31°N) and Cape Yubi (27°N)	30°50,7N/10°05,9E	355m for *B. marg.*; 800 to 1275m for *B. acul.*	13.5°C	35.75		very low sand content (less than 15%)

Appendix 2.1 *(Continued)*

Authors	Year	Species	Study area	Localisation	Occurrence Water depth	Bottom Water Temperature	Bottom Water Salinity	Microhabitat	Sediment type
Langezaal et al.	2006	*B. marginata*	the outer shelf of the Bay of Biscay	43°42?93''N/1°43?10"W	150m	11.9°C	35.6		
Mojtahid et al.	2006	*B.marginata* / *B. aculeata*	Outer continental shelf off Congo	5°16.50S/11°33.75E	70-230m	14°C			
Szarek et al.	2006	*B. marginata*	Central part of the Sunda shelf, southwestern South China Sea	2°01.6?-5°32.4? N and 107°02.0?-110°32.4? E	109 to 226m	18.3-26.2°C			
Di Leonardo et al.	2007	*B. marginata* (dead) 1945-2000	Sicilian coastal zone seawards of the industrial area of Augusta and the Palermo Gulf	37°09'00 N/15°16'00 E	60-150m	14.9-16.2°C			
Koho et al.	2007	*B. marginata*	Nazaré Canyon, western Iberian continental margin	39°38'29"N/9°16'29''W	146m (upper canyon terrace)	13.3°C (ENACW)	?36.0		
Mojtahid	2007	*B. aculeata* (and few sp. of *B. marg.*)	Rhône prodelta	43.3°N and 4.8°E	40 to 100m	13°C (80m) to 18°C (20m)	38	"predominantly superficial" (ALD=1.6 & 1.3cm for *B. marg.* & *B. acul.*, resp.)	
Murgese and De Deckker	2007	*B. aculeata* (fossils)	Indian Ocean	22°07.74?S/113°30.11?E; 14°00.55?S/121°01.58? E; 8°27.35?S/127°53.83? E	1093, 2452 and 1802m				
Smart et al.	2007	*Bulimina* spp. (fossils)	Indian Ocean						
Abu-Zied et al.	2008	*B. marginata* (fossils)	Aegean Sea	38° 56? N/25° 00? E	430m	14°C			
Brückner and Mackensen	2008	*B.marginata* (Dead, very recent)	Southern flank of the Skagerrak	57°50.260 N/8°42.327 E and 58°01.920 N/9°37.172 E	285-420m	6°C (300m) and 5.7°C (400m)		Shallow infaunal	
Eichler et al.	2008	*B. marginata*	South Brazilian shelf	27° to 30°S	20-150m	13.5-19.5°C	35.51-35.81		
Mojtahid et al.	2008	*B. marginata*	Firth of Clyde, Scotland	55°40.27?N/5°4.05?W	58-178m	8°C	34		

Appendix 2.1 *(Continued)*

Authors	Year	Species	Study area	Localisation	Occurrence Water depth	Bottom Water Temperature	Bottom Water Salinity	Microhabitat	Sediment type
Panieri and Sen Gupta	2008	*B. marginata*	Blake Ridge Diapir on the U.S. Atlantic continental margin	32°29.619?N/76°11.471? W	2155m	3.6°C			
de Nooijer et al.	In press	*B. marginata / B. elongata*	Frisian Front, southern North Sea	between 53°20' and 54°00'N, and 4°30'E	30-45m	7-17°C	33-34		
Frezza and Carboni	In press	*B. marginata*	Continental shelf off the Ombrone River, southern Tuscany	42°22?29?-42°45?02? N and 11°01?10?-10°32?35? E	53-177m	13.8°C			clayey sediments
Pascual et al.	In press	*B. marginata*	Basque continental shelf	43°10'-43°40'N and 1°30'-3°20'W	80-150m	11.6-12.0°C			
Pucci et al.	Submit.	*B. marginata*	Off Ancona, central Adriatic Sea	43°42'03"N/13°37'15"E	35m	14.8°C *in situ* & 14°C in exp.	38 *in situ* & 38.5 in exp.	Surface and a deep maximum	
Sen Gupta and Machain-Castillo	1993	*B.marginata / B. aculeata*	Review						
Bernhard	1996	*B. marginata*	Review						
Bernhard and Sen Gupta	1999	*Bulimina* spp.	Review						
Van Der Zwaan et al.	1999	*B. marginata*	Review						
Martins and Gomes	2004	*B. marginata*	Review						

CHAPITRE 3

OPTIMISATION OF LABORATORY CONDITIONS TO OBTAIN REPRODUCTION AND GROWTH OF THE DEEP-SEA BENTHIC FORAMINIFER *BULIMINA MARGINATA*

C. Barras [1,2]*, E. Geslin [1], J.-C. Duplessy [2], F. Jorissen [1]

[1] Laboratory of Recent and Fossil Bio-Indicators (BIAF), UPRES EA 2644, Angers University, France, and Laboratory of Marine Bio-Indicators (LEBIM), Ile d'Yeu, France

[2] Laboratoire des Sciences du Climat et de l'Environnement (LSCE), CEA–CNRS-UVSQ, Gif-sur-Yvette, France

Paper submitted to Journal of Experimental Marine Biology and Ecology

CHAPITRE 3

OPTIMISATION OF LABORATORY CONDITIONS TO OBTAIN REPRODUCTION AND GROWTH OF THE DEEP-SEA BENTHIC FORAMINIFER *BULIMINA MARGINATA*

Abstract

In this study, we investigate the impact of different food types and temperatures on the reproduction and increase of test size of the deep-sea benthic foraminifer *Bulimina marginata* (*sensu lato*) in laboratory conditions. We performed reproduction experiments with adults of *Bulimina marginata* at different temperatures (6, 8, 10, 12, 14°C), fed with either *Chlorella* sp. (freeze-dried green algae) or *Phaeodactylum tricornutum* (fresh diatoms). The test growth rate of juvenile specimens of *Bulimina marginata* was measured in experiments performed (1) at constant temperatures with different quantities of green algae and different types of food (green algae and/or diatoms; fresh, frozen or freeze-dried), and (2) with the same food at four different temperatures (8, 10, 12 and 14°C).

Bulimina marginata is well adapted to laboratory conditions and can reproduce and grow in all conditions tested in our experiments, from 6 to 14°C and fed with green algae or diatoms, fresh, frozen or freeze-dried. Nevertheless, the results suggest that temperature has a great influence on the physiological processes of these deep-sea foraminifera: lower temperatures lead to delayed reproduction and less increase of test size of the juveniles. Experiments with fresh *Phaeodactylum* produced better results than experiments with freeze-dried *Chlorella*: the time before reproductions occurred was shorter, the number of juveniles produced per reproduction event was higher and the juveniles grew faster. These differences may be the consequence of the freeze-drying process, which alters the quality of the food that becomes less rich in digestible compounds (decrease of the carbohydrates and polyunsaturated fatty acids contents). When only freeze-dried *Chlorella* was chosen as food particles, several food additions yielded to higher test growth rates than one single addition. The fact that reproduction and growth performances of *Bulimina marginata* strongly depend on the food quality confirms the opportunistic nature of this taxon.

Key words

Asexual reproduction; *Bulimina marginata*; Freeze-dried food; Temperature; Test growth

1. INTRODUCTION

Benthic foraminifera are single-celled organisms, widespread on the seafloor of all oceans (Gooday *et al*., 1992); they have the particularity to build a test with a high fossilising potential. Foraminifera are frequently used as tools for paleoenvironmental reconstructions, which are either based on the composition of the faunas (e.g. Jorissen *et al*., 2007) or on the geochemical composition of their shell ($\delta^{18}O$, $\delta^{13}C$, trace metals, etc.). Calibrations of these proxies have been obtained by inorganic precipitation of calcite or by comparisons of the geochemical composition of the foraminiferal tests with *in situ* measurements or literature data of the target parameters, such as temperature, salinity, bottom water oxygenation or the flux of organic matter to the ocean floor. Today, an increasing number of researchers are developing laboratory cultures of benthic foraminifera to better calibrate these paleoceanographical proxies (e.g. Chandler *et al*., 1996; Wilson-Finelli *et al*., 1998; Toyofuku *et al*., 2000; Havach *et al*., 2001; Hintz *et al*., 2006a, 2006b). In comparison to field studies, the advantage of these laboratory experiments is that they allow us to understand the influence of a single parameter while all other physico-chemical (pH, salinity, temperature, etc.) or geochemical parameters ($\delta^{18}O$, $\delta^{13}C$, trace metals, etc.) can be kept stable. Numerous laboratory experiments aimed to study the impact of environmental parameters (e.g. food availability, temperature, salinity, pollution…) on the behaviour of benthic foraminifera, from shallow water (Bradshaw, 1955, 1957, 1961; Murray, 1963; Alve and Bernhard, 1995; Stouff *et al*., 1999a, 1999b, 1999c; Nigam and Caron, 2000; Alve and Goldstein, 2002; Heinz *et al*., 2005; Ernst *et al*., 2002, 2005, 2006; Le Cadre and Debenay, 2006; de Nooijer *et al*., 2007) as well as from deep sea ecosystems (Hemleben and Kitazato, 1995; Gross *et al*., 2000; Heinz *et al*., 2001, 2002; Geslin *et al*., 2004; Nomaki *et al*., 2005a). On the contrary, laboratory experiments with deep-sea benthic foraminifera aiming at a quantitative calibration of geochemical proxies are still very scarce (Wilson-Finelli *et al*., 1998; Havach *et al*., 2001; Hintz *et al*., 2006a, 2006b; McCorkle *et al*., 2004).

In this paper, we concentrate on the *Bulimina marginata* group (d'Orbigny, 1826). *Bulimina marginata* is a cosmopolitan species, which has been described in different environments such as

the Atlantic Ocean (e.g. Lutze and Coulbourn, 1984; Corliss, 1991; Jorissen *et al*., 1998; Fontanier *et al*., 2002; Mendes *et al*., 2004; Eberwein and Mackensen, 2006; Langezaal *et al*., 2006; Koho *et al*., 2007; Mojtahid *et al*., 2006, 2008; Panieri and Sen Gupta, 2008; Eichler *et al*., in press; Pascual *et al*., in press), the Mediterranean Sea (e.g. Jorissen, 1988; Barmawidjaja *et al*., 1992; De Rijk *et al*., 2000; Bergin *et al*., 2006; Di Leonardo *et al*., 2007; Abu-Zied *et al*., 2008; Frezza and Carboni, in press), the North Sea (e.g. Brückner and Mackensen, 2008; de Nooijer *et al*., in press), the Pacific Ocean (e.g. Phleger and Soutar, 1973) and the China Sea (Szarek *et al*., 2006). In certain environments, a wide morphological variation may be found, even at a single site. For this reason, Jorissen (1988) distinguished three morphotypes (*marginata*, *denudata* and *aculeata*) within *B. marginata*. Other authors (e.g. Collins, 1989; Burgess and Schnitker, 1990) have considered the extremes of the morphological variation as separate species (*B. marginata* and *B. aculeata*). There is still no conclusive biological/genetical evidence showing whether *B. marginata* is a cryptic species, or rather a single species with a large ecophenotypical variability. As we explained in Chapter 2, we will consider here two morphotypes *B. marginata* f. *marginata* and *B. marginata* f. *aculeata*.

Unlike many other deep-sea foraminiferal taxa, *Bulimina marginata* (*sensu lato*) previously showed a good adaptation to laboratory conditions (Wilson-Finelli *et al*., 1998; Havach *et al*., 2001; Bernhard *et al*., 2004; Hintz *et al*., 2006a, 2006b; McCorkle *et al*., 2004). Whereas many other taxa remain inactive and ultimately die in laboratory conditions, Wilson-Finelli *et al*. (1998) and Havach *et al*. (2001) observed reproduction and obtained juveniles of *B. marginata* in sediment micro- and mesocosms.

The possible use of a deep-sea benthic foraminiferal taxon for the laboratory calibration of paleoceanographical proxies depends on the survival, reproduction, growth and calcification of the deep-sea foraminifera in the laboratory. Environmental parameters such as temperature and food regime are known to have a decisive influence on these physiological processes and on the spatial distribution of foraminifera in the ocean. Earlier, Bradshaw (1955, 1957, 1961) and Bijma *et al*. (1990) studied the influence of temperature on reproduction, test growth rate, survival and/or feeding rate of shallow benthic and planktonic foraminifera, respectively. It is obvious that the availability of suitable food is also of importance for activity, growth and maintenance of other life processes. Foraminifera have been reported to have different feeding strategies; they may be herbivores, detritivores, carnivores or omnivores (review in Murray, 1991; Goldstein, 1999). In

various areas it has been demonstrated that foraminiferal standing stocks are dependant on organic matter fluxes to the sea floor (e.g. Lutze and Coulbourn, 1984; Jorissen *et al*., 1995; Schmiedl *et al*., 2000; Fontanier *et al*., 2002). *Bulimina marginata* is commonly found in eutrophic areas where the organic matter content of the sediment is high (e.g. Lutze and Coulbourn, 1984; Jorissen *et al*., 1998; Schmiedl *et al*., 2000; Fontanier *et al*., 2002; Eberwein and Mackensen, 2006; Hayward, 2007). De Rijk *et al*. (2000) concluded that this species is one of the most opportunistic taxa found in the Mediterranean. According to Eberwein and Mackensen (2006), *Bulimina marginata* tends to be associated with areas of maximal surface-water chlorophyll-*α* concentrations (elevated organic matter supply to the sea floor), whereas *B. aculeata* occurs when conditions are poorer. In a study on the evolution of the eastern Indian Ocean over the last 30 kyrs, *B. marginata* has been used as an indicator of increased organic matter supply to the sea floor (Murgese and De Deckker, 2007).

The aim of this study is to optimise the experimental setup to culture deep-sea benthic foraminifera by (1) investigating the influence of food sources and temperature on physiological processes (reproduction of adults and growth of juveniles) of *Bulimina marginata*, and (2) determining the favourable conditions to obtain, in a short period of time, reproduction and test growth of this species in laboratory conditions. Our paper describes a series of experiments carried out over a period of two years. We think that our experimental results will be particularly useful for the many groups which presently try to set up culture experiments of deep sea foraminifera.

2. MATERIAL AND METHODS

The living specimens of benthic foraminifera used in our experiments were sampled at two stations in the Bay of Biscay, north-east Atlantic, in the axis of Cap Breton canyon. Stations G (43°40'N-1°37'W) and K (43°37N-1°43W) are respectively situated at 450 and 650 m water depth. The foraminiferal assemblages at these two locations are strongly dominated by *Bolivina subaenariensis* and *Bulimina marginata* (Hess *et al*., 2005; Hess and Jorissen, in press).

At both stations, several cores were taken with a multi-tube corer (Barnett *et al*., 1984) in August 2005 and June 2006. The top 2 centimetres, containing the largest number of living foraminifera, were kept in the laboratory under atmospheric pressure, in seawater at 10°C, at a salinity of 36‰, without addition of food. This sediment constituted a stock of living foraminifera that could be used for the experiments even after a relatively long period of time (4 to 10 month). In fact, faunal

density decreases over time but *Bolivina subaenariensis* and *Bulimina marginata* still present significant amounts of living specimens after such periods of incubation.

2.1. Experimental conditions

2.1.1. Temperature

At both sampling stations, the *in situ* temperature is about 10°C. Temperatures chosen for the experiments ranged from 6 to 14°C; by choosing a temperature range around the value observed in the field, we hoped that the faunas would not be disturbed by the temperature difference. To carry out experiments at different temperatures, incubators were maintained at 6, 8, 10, 12 and 14°C. The stability of the incubators was monitored with thermometers (Testo-174 or 175-T2), which recorded the temperature inside the incubators every 10 minutes during the entire duration of the experiments.

2.1.2. Food

Two different strains of green algae were used as food for foraminifera: *Chlorella* sp. and *Dunaliella tertiolecta*. *Chlorella* sp., obtained from AQUAMER S.A. (France), is sessile and was in our experiments used in a freeze-dried form. This strain has previously been used as food for benthic foraminifera by Heinz *et al*. (2001) in freeze-dried form and by Toyofuku *et al*. (2000) in fresh form mixed with diatoms. Also Gross (2000) demonstrated that this algae may successfully be used as food source for deep-sea benthic foraminifera. *Dunaliella tertiolecta,* provided by the University of Tübingen (Germany), is a planktonic algae cultured in the laboratory. *Dunaliella tertiolecta* was tested by several authors in laboratory cultures, either mixed with other phytoplanktonic algae (e.g. Chandler *et al*., 1996; Wilson-Finelli *et al*., 1998; Havach *et al*. 2001; Heinz *et al*., 2001, 2002; Bernhard *et al*., 2004; Nomaki *et al*. 2005a) or in monospecific diets (Bradshaw, 1961; Hintz *et al*., 2004).

We cultured also two strains of diatoms. *Phaeodactylum tricornutum,* obtained from CCAP (UK), has a golden pigmented colour. Since this species occurs in planktonic as well as benthic communities (Round *et al*., 1990), it may be in suspension in the medium or lie at the bottom of the culture vessel. These diatoms have previously been used as a food source for foraminiferal culture experiments by Murray (1963), Wilson-Finelli *et al*. (1998), Havach *et al*. (2001) and Le

Cadre and Debenay (2006), either as a monospecific diet, or mixed with other algae. Finally, also *Amphiprora alata* (CCAP, UK), a sessile diatom, has been used previously for laboratory experiments with benthic foraminifera (Stouff *et al*., 1999a, 1999b, 1999c; Heinz *et al*., 2001, 2002; Nomaki *et al*., 2005a; Le Cadre and Debenay, 2006).

All cultures of algae and diatoms took place at 20°C with a 12h/12h light-dark cycle. To maintain cultures in a phase of exponential growth, the culture medium was renewed every 15 to 20 days. All the cultures were resuspended before sampling or changing the medium. *Dunaliella tertiolecta* grew in a F/2 medium (Guillard and Ryther, 1962) in 500 ml Erlenmeyer flasks, and *Phaeodactylum tricornutum* and *Amphiprora alata* in a F/2 medium with addition of sodium metasilicate, respectively in Erlenmeyer flasks and in glass Petri dishes.

The interest to use freeze-dried algae (in our study, only *Chlorella*) is that it was not necessary to simultaneously maintain algal cultures, which is a time consuming occupation. Furthermore, it was easier to add exactly a precise amount of food to the experimental jar. *Chlorella* was weighed before addition to the foraminiferal cultures. Cultured diatoms and algae were added fresh and their quantity was measured in millilitres of added medium plus algae. However, the population dynamics of these cultures, with varying standing stocks, implies that the exact quantity of cells added to the experiment was not exactly the same from one addition to another. In order to limit this problem, and to add fresh algae/diatoms in a similar stage of population dynamics, we systematically added them around 15 days after changing their culture medium.

All experiments were carried out without sediment, and this for two main reasons: (1) to facilitate the observations under the stereomicroscope and (2) to prevent foraminifera from living in the sediment where the geochemical conditions may vary and are much more difficult to control.

2.2. Protocols for reproduction experiments

The principle of the reproduction experiments was to maintain adult specimens of *Bulimina marginata* in different conditions with respect to food and temperature in order to evaluate the impact of these different conditions on the frequency and success of reproduction. This paper presents four laboratory reproduction experiments that were performed at different times over a two years period. The laboratory protocol evolved over time, although the essentials remained

relatively similar. The experiments are presented here in a chronological order, from experiment A to experiment D. It should be noticed that no replicate experiments were performed due to extremely time-consuming character of the experiments.

Each experiment comprises four jars placed at different temperatures (range from 6 to 14°C) always with the same diet for each experiment. In experiment C, on the contrary, only one culture jar was kept at 10°C (Table 3.1). Two different food sources were tested in these experiments: freeze-dried *Chlorella* and fresh *Phaeodactylum*. Table 3.1 summaries the particular culture conditions for the four reproduction experiments. The total quantity of food added during the experiments is indicated as well as the number and timing of the food additions.

Experi-ments	Culture jars	Number of adults of *Bulimina*	Temperature (°C)	Food type	Total quantity of food	Number of food addition	Experiment duration (days)
A (4 jars)	A-Chlo6 A-Chlo8 A-Chlo10 A-Chlo14	25	6 8 10 14	freeze-dried *Chlorella* sp.	12 mg	3 (at t=0, 43 and 61 days)	101
B (4 jars)	B-Chlo6 B-Chlo8 B-Chlo10 B-Chlo12	26 = 5 (t=0) + 6 (t=56 days)+ 15 (t=60 days)	6 8 10 12	freeze-dried *Chlorella* sp.	7 mg	2 (at t=0 and 63 days)	97
C (1 jar)	C-Phaeo10	50	10	fresh *Pheaodactylum tricornutum*	8 ml	4 (at t=0, 11, 21 and 29 days)	50
D (4 jars)	D-Phaeo8 D-Phaeo10 D-Phaeo12 D-Phaeo14	28	8 10 12 14	fresh *Pheaodactylum tricornutum*	6 ml	2 (at t=0 and 18 days)	87

Table 3.1: Experimental conditions for the reproduction experiments with B. marginata *(experiments A, B, C and D).*

To be able to distinguish between living and dead foraminifera, adult specimens of *B. marginata* were picked from the bulk sediment and then stored for one or two days in a Petri dish containing *Chlorella*. The living and active foraminifera ingested the algae and consequently, their cytoplasm became green. This technique to determine living specimens was only adopted after the end of the first experiment; it was not yet used for experiment A. For experiments B, C and D, 26 to 50 intensely green-coloured adult specimens of *B. marginata* were added to each culture jar. For experiment A, 25 adults that appeared filled with cytoplasm were selected from the bulk sediment. For experiment B, the adults have been added in three times because of the poverty of

B. marginata in the bulk sediment (Table 3.1). Living foraminifera were always picked on a tray of ice to maintain cool conditions.

For all the experiments we used 60 or 120 ml plastic crystallising jars. The culture medium for benthic foraminifera was microfiltrated (at 0.45 µm) natural surface seawater from the Bay of Biscay, which is characterised by a salinity of 36‰, a pH of 8.1 and an alkalinity of about 2300 $\mu mol.l^{-1}$. In experiments B and D, where we renewed the seawater every 4 days, the physico-chemical conditions were very stable. In experiments A and C, the maximum variation observed between the start and the end of the experiments was a 1‰ increase in salinity and a drop in pH of 0.4. The experiments lasted from 50 to 101 days (Table 3.1) and observations of the foraminifera were made every 3 to 10 days.

It is important to keep in mind that the culture jars of each single experiment are fully comparable since: (1) the adult specimens of *B. marginata* were sampled from the same bulk sediment sample, (2) the food quantity and quality was exactly the same, and (3) the methodology employed was exactly the same. Therefore, the outcome of each experiment provides us reliable information about the influence of temperature on reproduction of adult specimens, fed with *Chlorella* or *Phaeodactylum*. Comparisons between different experiments can inform us about the influence of different diets. However, these comparisons must be considered with some reserve, since the experimental protocol (the quantity of food in particular) was slightly modified in the two years during which the experiments were performed.

2.3. Protocols for growth experiments

Juvenile specimens of *Bulimina marginata* (*marginata* and *aculeata* morphotypes) used in the test growth experiments were labelled using the fluorescent compound calcein (Bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) (Bernhard *et al*., 2004). This compound binds to calcium in biomineralized structures. This calcein-tagging method allows to discriminate between pre-existing foraminiferal calcium carbonate and calcite precipitated during the experimental treatments.

The larger fraction (> 150 µm) of sediment samples (top two centimetres of sediment cores sampled in the field) containing adult foraminifera was incubated in a 10 $mg.l^{-1}$ solution of calcein (Fluka, Sigma Aldrich) in microfiltrated seawater in a Duran bottle at 10°C (*in situ* temperature). All the calcite formed during the incubation period showed a yellow-green fluorescence when viewed under an Olympus SZX-12 stereomicroscope equipped with epifluorescence optics

(excitation at 470 nm, emission at 500 nm). Calcite precipitated after calcein exposure did not fluoresce, and therefore all calcite formed in experimental conditions was easily identifiable (Plate 3.1, Fig. A-2). Bernhard *et al*. (2004) demonstrated that the incubation in calcein does not have a negative effect on foraminiferal calcification or survival at the concentration we used; in their study, specimens of *Bulimina aculeata* incubated in calcein (10 $mg.l^{-1}$) showed a similar survival rate as control specimens (Bernhard *et al*., 2004). The fluorescent tag is permanent and is also observable in archived specimens.

After one to three months of calcein incubation of the larger fraction of the sediment, fed with some *Chlorella* (change of the calcein solution on the average once a month), the sediment was sieved over 150 and 38 µm meshes with seawater at 10°C. If reproductions of mature specimens happened during the incubation period, juveniles were present in the 38-150 µm fraction (Plate 3.1, Fig. A-1). Juvenile specimens of *marginata* and *aculeata* morphotypes were mostly found together in the small fraction. Mixed assemblages of both morphotypes were used for growth experiments since it was very difficult to differentiate between these two morphotypes for the most juvenile specimens (2 or 3 chambers). Nevertheless, we estimated the amount of *marginata* and *aculeata* morphotypes. In experiments I to V, *marginata* morphotypes strongly dominated the assemblage (94 ± 6%). Only in experiment VI, a larger amount of *aculeata* morphotypes was introduced (25 ± 2%). They were picked under the epifluorescent stereomicroscope, with a fine brush or small-bore mouth pipette, on a tray of ice to maintain cool conditions.

All the test growth experiments took place in plastic crystallising jars (60 ml), covered with a lid, and completely filled with microfiltrated (0.45 µm) natural seawater from the Bay of Biscay (same characteristics as the medium used in the reproduction experiments). Experiments I to V (15 jars) were designed to determine the influence of different food sources on the test growth of the juveniles at a temperature of 10°C (*in situ* temperature). We tested (1) different quantities of freeze-dried green *Chlorella* algae (experiment I, jars C_1, C_2 and C_3); (2) different types of food: freeze-dried green *Chlorella* algae and fresh *Phaeodactylum* diatoms (experiment II, jars C, C+P and P, and experiment III, jars C, C+P and P), fresh *Phaeodactylum* and *Amphiprora* diatoms (experiment IV, jars P and A); and (3) different states of the food: fresh or frozen for green *Dunaliella* algae and *Phaeodactylum* diatoms (experiment V, jars D_1, D_2, P_1 and P_2) (Table 3.2). Experiment VI (jars A8, A10, A12 and A14) aimed to test the influence of temperature, using always the same diet (fresh *Amphiprora*). Table 3.2 summarises the conditions of feeding and

temperature for all the experimental setups, and indicates the number of juveniles of *Bulimina marginata* (*marginata* and *aculeata* morphotypes) added to the crystallising jars. On average, all experiments lasted one month, with the exception of experiment VI that lasted only 20 days (Table 3.2). Only one experimental condition has been replicated (I-C and II-C) since performing replicate experiments for all the conditions was in practice not realisable due to time limitation. However, the number of juveniles added per experiment jars was maximized (more than 60 specimens) in order to obtain statistically reliable results.

Experi-ments	Culture jars	Number of juveniles of *Bulimina*	Temperature (°C)	Food type	State of the food	Quantity of food	Number of food addition	Experiment duration (days)
I (3 jars)	$I\text{-}C_1$	75	10	*Chlorella*	freeze-dried	2 mg	1	31
	$I\text{-}C_2$	71		*Chlorella*	freeze-dried	4 mg		
	$I\text{-}C_3$	67		*Chlorella*	freeze-dried	8 mg		
II (3 jars)	II-C	61	10	*Chlorella*	freeze-dried	2 mg	1	28
	II-C+P	63		*Chlorella* + *Phaeodactylum*	freeze-dried + fresh	1 mg + 1 ml		
	II-P	63		*Phaeodactylum*	fresh	2 ml		
III (3 jars)	III-C	66	10	*Chlorella*	freeze-dried	2 mg	2	29
	III-C+P	68		*Chlorella* + *Phaeodactylum*	freeze-dried + fresh	1 mg + 1 ml		
	III-P	78		*Phaeodactylum*	fresh	2 ml		
IV (2 jars)	IV-P	79	10	*Phaeodactylum*	fresh	1 ml	4	34
	IV-A	71		*Amphiprora*	fresh	1 ml		
V (4 jars)	$V\text{-}D_1$	81	10	*Dunaliella*	fresh	3 ml centrif.	2	35
	$V\text{-}D_2$	80		*Dunaliella*	frozen	1 ml		
	$V\text{-}P_1$	82		*Phaeodactylum*	fresh	3 ml centrif.		
	$V\text{-}P_2$	80		*Phaeodactylum*	frozen	1 ml		
VI (4 jars)	VI-A8	97	8	*Amphiprora*	fresh	3 ml + 1.5 ml	2	20
	VI-A10	93	10					
	VI-A12	95	12					
	VI-A14	102	14					

Table 3.2: Experimental conditions for the growth experiments with B. marginata *(experiments I to VI).*

We measured the size of the specimens added at the start of the experiments as well as the size of specimens at the end of each experiment. Length measurements were made from the proloculus to the top of the last calcified chamber (e.g. Plate 3.1, Figs. A-1 and 3). These measurements were performed using the microscale incorporated in the ocular of the stereomicroscope (LEICA MZ 125). During these measurements, we used always the same magnification and the ocular microscale was calibrated using a slide microscale with a precision of 10 µm.

Two different starting pools of juveniles were used for experiments I to IV (pool 1) and for experiment V (pool 2). To evaluate the size of the juveniles added in the culture jars, 100 specimens were picked randomly from each starting pool and were measured. For jars of experiments I, II, III and IV, the average initial size of the juveniles was 93 ± 12 µm (pool 1). For jars of experiment V, added juveniles had a size of 158 ± 32 µm (pool 2) at the beginning of the

experiments. A third pool of juveniles was used for experiment VI. The test growth rate was calculated for each specimen taking into account the average length of the juveniles added at the beginning of the experiment (average size of the specimens of pool 1 or 2) and the length of the specimens after one month of experiment (Appendix 3.1) according to the following equation:

$$\textit{Test growth rate (\% per month)} = [(\textit{final size} - \textit{initial size}) / \textit{intial size}] * 100$$

The mean test growth rate (with standard deviation) per experiment jar has been based on all calcifying specimens; non calcifying specimens have not been taken into account.
A student's *t*-test was used to identify significant differences between average test growth rates obtained in the different experiments. The null hypothesis, i.e. no significant difference between two average values, is accepted if the calculated *t*-value is inferior to the critical value of *t* for a given degree of freedom (*Df*) and confidence level (*p*), otherwise the null hypothesis is rejected, and the difference is considered significant.

Since we worked with only one species, length measurements are an adequate way to describe the rate of test size increase of the specimens. However, it is also interesting to know the increase of foraminiferal biovolume, which can be estimated on the basis of the increase in length and width of the individuals (Plate 3.1, Figs. A-1 and 3; Appendix 3.1). The common method to determine foraminiferal biovolume is to measure the volume of the test (e.g. Altenbach, 1987; Hannah *et al*., 1994). Test volume was approximated by calculating the volume of an ellipsoid (Plate 3.1, Figs. A-1 and 3). Although the shape of an adult *Bulimina marginata* resemble to a cone, juvenile specimens look more like spheres. Since we have a mix of both juvenile and adult specimens, we decided to estimate the foraminiferal volume with the following formula:

$$\textit{Test volume } (\mu m^3) = 4/3 * \pi * (\textit{Length} / 2) * (\textit{Width} / 2)^2$$

Normally, the volume corresponding to the test wall should be subtracted from the total volume of the test. However, since our measurements were not performed with a scanning electron microscope, the precision of our measurements (10 µm) is not high enough to have a precise idea of the thickness of the wall. Another problem is that the foraminiferal cytoplasm does not entirely fill the test (Gerlach *et al*., 1985; Hannah *et al*., 1994). Following Hannah *et al*. (1994), we assumed that the foraminiferal biovolume represents 75% of total test volume. On the basis of the average initial biovolume of the introduced juveniles and the average biovolume of the

foraminifera at the end of each experiment, we calculated a biovolume growth coefficient ($R_{biovolume}$):

$$R_{biovolume} = Final\ biovolume / Initial\ biovolume.$$

3. RESULTS

3.1. Reproduction experiments

3.1.1. General observations

Visual observations of all reproduction experiments, allowing us to study the behaviour of adult specimens of *B. marginata,* were made regularly, every 3 to 10 days. These observations were made for all temperatures and food regimes. When *Chlorella* or *Phaeodactylum* was added to the culture medium, active specimens ingested these food particles and the colour of the cytoplasm became similar to that of the food. After only one night, active adult specimens of *B. marginata* turned green in the presence of *Chlorella* and brown in the presence of *Phaeodactylum*. Some of the specimens accumulated food particles around their aperture, thus constituting a so-called feeding cyst (Goldstein, 1999). In our experiments, living adult specimens were often in a vertical position, with their aperture against the bottom of the crystallising jar. Specimens were moving in the crystallising jars and some climbed on the walls of the culture jars.

3.1.2. Observation of reproduction events

The observations of reproduction described in this paragraph were similar for all our experiments with *B. marginata*. The successive steps of the reproduction process are summarised in Figure 3.1. Just before reproduction, adult specimens gathered a large amount of food around their test, much more than was usually accumulated around the aperture (in the so-called feeding cyst). When surrounded by such a "reproduction cyst", the foraminiferal test itself became in many cases almost invisible. Nevertheless, because of this excess food gathering, mature specimens, ready to reproduce, could easily be recognised. Reproduction itself generally took place several days to one week after the production of the reproduction cyst. Part of the juveniles released by the adult was found stuck in the reproduction cyst, whereas the others were spread around the adult that reproduced (Figure 3.1). Plate 3.1, Fig. B-1 gives a typical picture of this situation: a single dead adult specimen is present in the middle of an area covered with juveniles, whereas the remainder of

the reproduction cyst still contains juveniles. These reproduction events happened at the bottom of the crystallising jars but also on their side walls. After the reproduction events, the adults always died, and were separated from the reproduction cyst containing the juveniles (Plate 3.1, Fig. B-2).

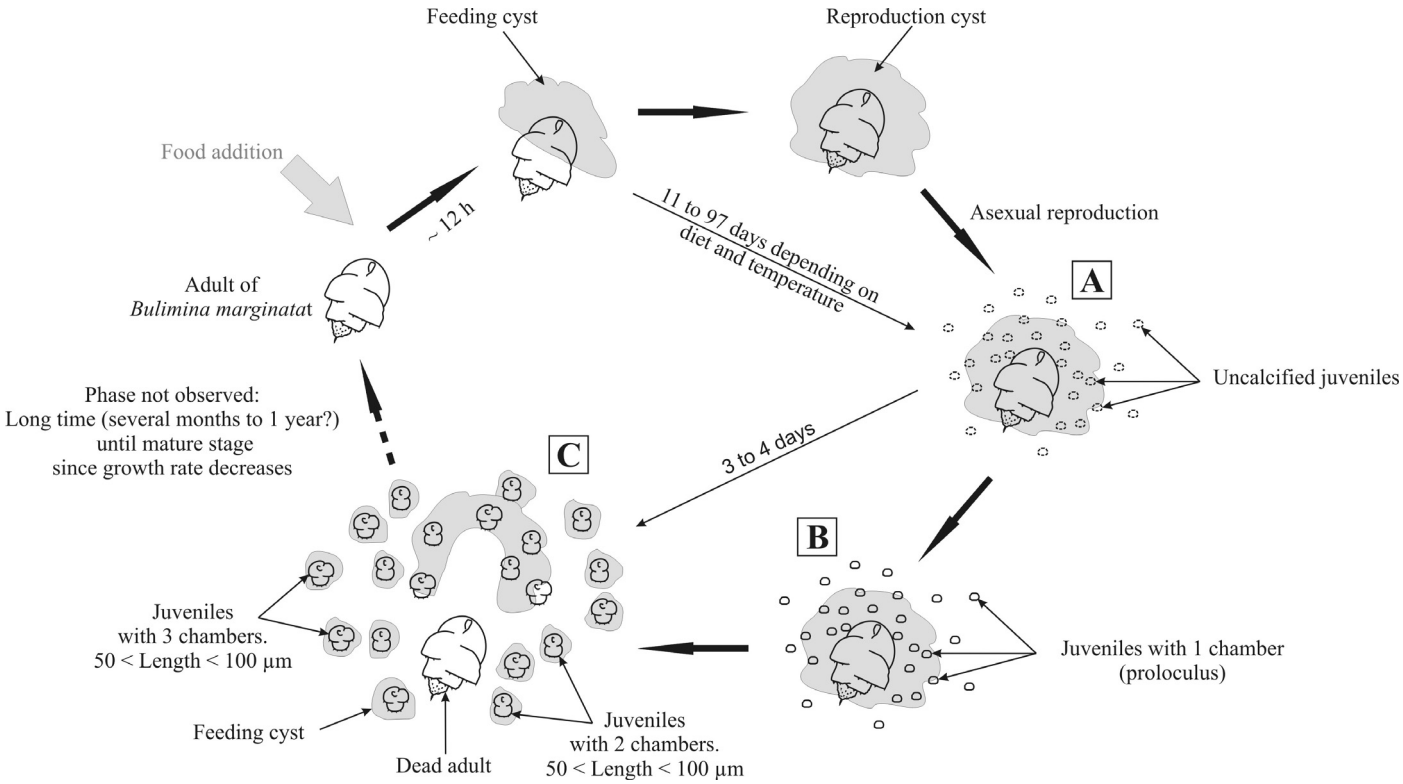


Figure 3.1: Different steps observed during the asexual reproduction of B. marginata *and the early stages of juvenile chamber growth. A: Production of the uncalcified juveniles, B: calcification of the proloculus, and C: calcification of the 2-3 first chamber 3 to 4 days after the release of the uncalcified juveniles.*

Plate 3.2, Fig. A-1 shows a typical adult specimen, such as those used in the reproduction experiments. Rather surprisingly, the adults that reproduced often revealed irregularities in the chambers calcified just before the reproduction event, such as the presence of a broken chamber (Plate 3.2, Figs. A-2 and 3), abnormal chamber shape (Plate 3.2, Figs. A-2 and 3), or aperture anomalies such as double apertures located in two different chambers (Plate 3.2, Fig. A-4) or in a single chamber (Plate 3.2, Fig. A-5). One adult specimen that calcified its last chamber against the glass of the crystallising jar was observed after reproduction with a juvenile specimen still present in the last chamber (Plate 3.2, Fig. A-6).

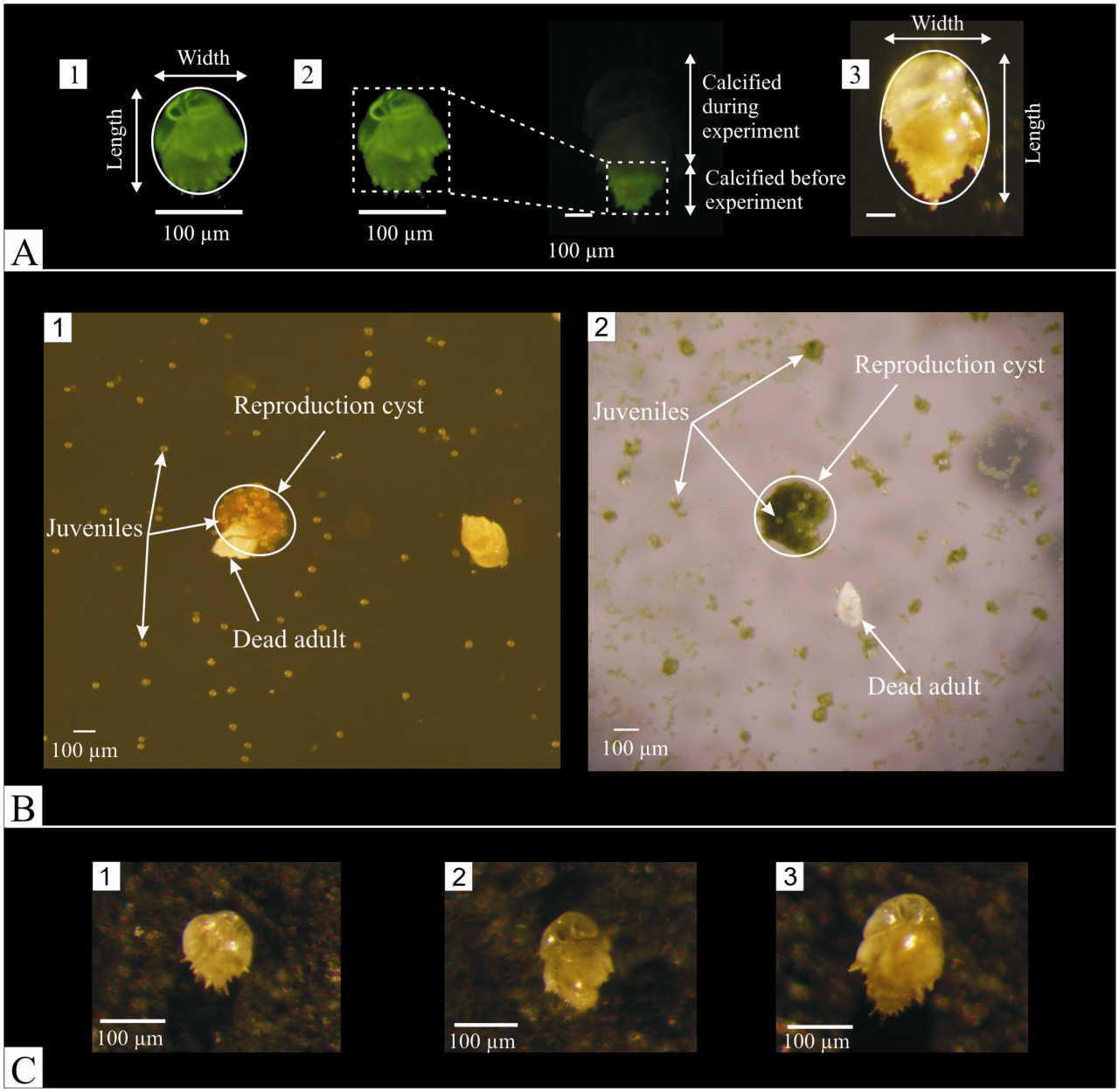


Plate 3.1: Fig. A-1: Juvenile of B. marginata *that is born in calcein bath (epifluorescent stereomicroscope picture); Figs. A-2 and 3: Specimen marked with calcein (A-2, epifluorescent stereomicroscope picture) that calcified new chambers in culture (A-3, stereomicroscope picture); Figs. B-1 and 2: Stereomicroscope pictures of the reproduction of* B. marginata *in the presence of fresh* Phaeodactylum *(B-1) and freeze-dried* Chlorella *(B-2); Fig. C: Pictures of typical specimens obtained after one month of growth in the experiment II: Fig. C-1 with a diet of* Chlorella *(II-C), Fig. C-2 with a mix of* Chlorella *and* Phaeodactylum *(II-C+P), and Fig. C-3 with a diet of* Phaeodactylum *(II-P). Note the difference of size between the specimens and the difference in the shape of their shell with sharper undercut margins in C-2 and 3 than in C-1.*

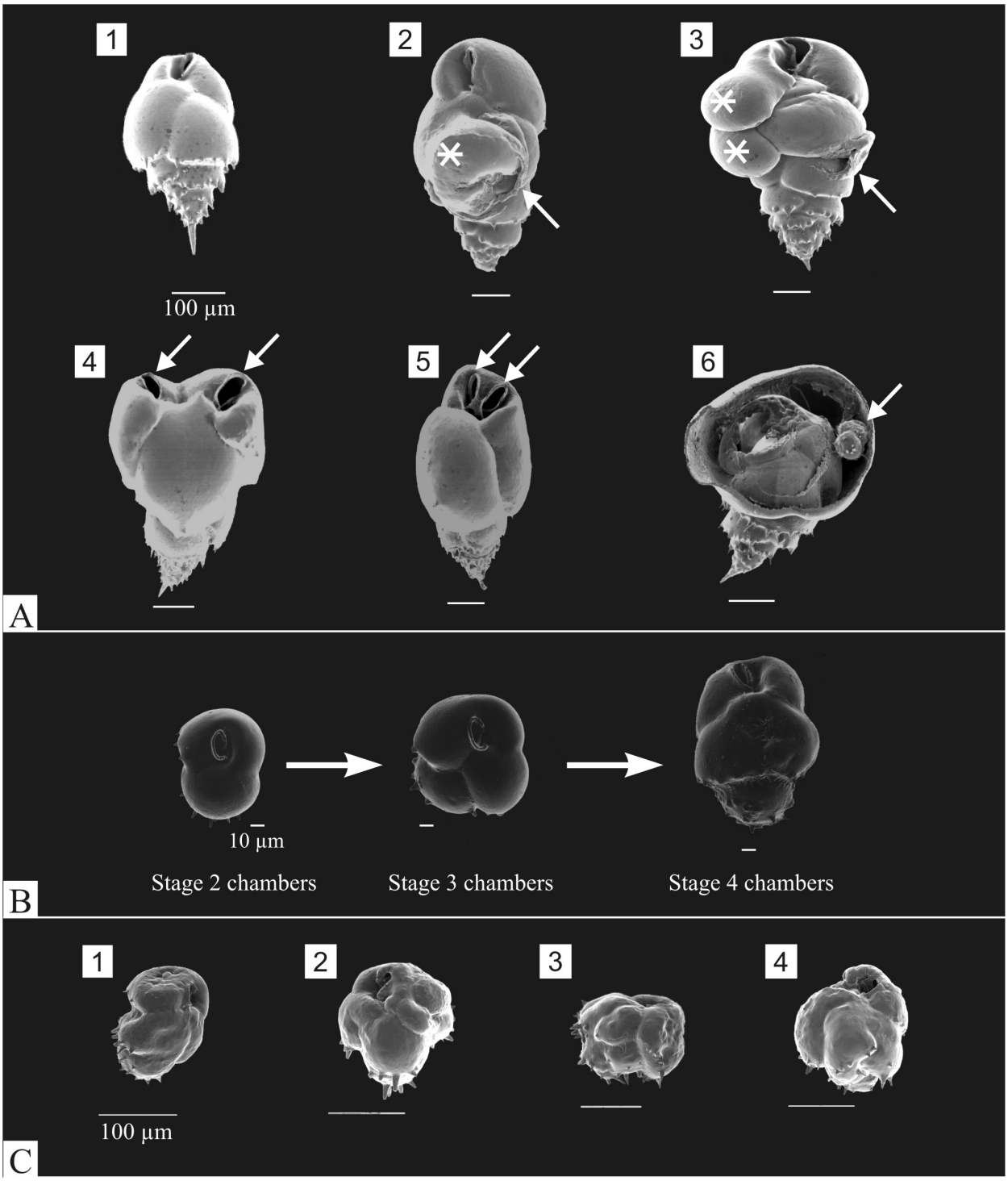


Plate 3.2: Fig. A-1: Typical adult specimen of B. marginata *added in the reproduction experiments (SEM picture); Figs. A-2 to 6: Specimens that supposedly reproduced which show morphological anomalies indicated by arrows, such as broken chambers (A-2 and 3), double aperture either in 2 separate chambers (A-4) or in a single chamber (A-5), or by stars, such as abnormal chambers (A-2 and 3); Fig. A-6: specimen that calcified its last chamber against the jar wall with a juvenile stuck inside; Fig. B: First isolated growth stages of* B. marginata *(stage 2, 3 and 4 chambers); Figs. C-1 to 4: Juvenile specimens produced in the reproduction experiments which present test anomalies affecting test wall and/or shape.*

3.1.3. Observation of the early stages of juvenile chamber growth

Repeated examination of the experiment jars under the stereomicroscope provided information about the successive ontogenetic stages of the juveniles. Just after the reproduction of the adults, the juveniles released in the surrounding environment look like cytoplasm balls without a calcified wall (Figure 3.1-A). The calcification of the proloculus (the first chamber, Figure 3.1-B) and of the second and third chamber happened very quickly (Figure 3.1-C), within 3 to 4 days. Since observations were only made every 3 to 10 days, these first stages could not always be observed. After the calcification of the two next chambers (chambers 3 and 4, total size about 100-150 µm), the growth of the juveniles became much slower. Specimens with 2, 3 and 4 chambers are shown on Plate 3.2, Fig. B. All the juveniles produced in our reproduction experiments present a megalospheric proloculus, showing that they are the result of asexual reproduction.

Test anomalies were sometimes observed in the juveniles specimens sampled at the end of the experiments. These anomalies affected the test wall and sometimes also the shape of the test. The surface of the shell walls appears to be much rough and bumpy in the juveniles than in adult specimens (Plate 3.2, Figs. C-1, 2, 3 and 4).

3.1.4. Quantitative results of the experiments

Table 3.3 summarises the culture conditions as well as the quantitative observations made during the reproduction experiments. Reproductions occurred in all the tested conditions of temperature and food type. *Bulimina marginata* can reproduce in the laboratory with *Chlorella* or *Phaeodactylum* as food particles and from 6 to 14°C, without sediment (Table 3.3). In Table 3.3, the time before the first reproduction occurred is indicated (11 to 97 days, according to temperature and food conditions) as well as the total number of specimens produced at the end of the experiment (21 to 669 juveniles). Because the number of adults added at the beginning of the experiments was not always the same, we calculated the average number of juveniles produced per adult added in the culture jars ($R_{juveniles/adults}$). Whenever possible, the number of juveniles produced per single reproduction event was counted (Table 3.3). When several reproduction events occurred simultaneously in one crystallising jar, the number of juveniles of each single reproduction could not be determined because juveniles from different reproduction events mixed. It was also impossible to count the juveniles when reproduction took place on the side walls of the crystallising jars. To avoid any perturbation of the fauna, foraminifera were not moved or touched.

Therefore, also the number of juveniles remaining in the reproduction cysts could not be counted precisely.

Culture jars	Number of adults of *Bulimina*	Temperature (°C)	Food type	Experiment duration (days)	Time before first reproduction *(previous observation)* (days)	Total number of juveniles	$R_{juveniles/adults}$	Number of juveniles per reproduction
A-Chlo6	25	6	freeze-dried *Chlorella*	101	*(61-)* **68**	158	6.3	30
A-Chlo8	25	8	freeze-dried Chlorella	101	*(24-)* **34**	243	9.7	40
A-Chlo10	25	10	freeze-dried *Chlorella*	101	*(24-)* **34**	245	9.8	21
A-Chlo14	25	14	freeze-dried *Chlorella*	101	*(24-)* **34**	161	6.4	
B-Chlo6	26 = 5(t=0) + 6 (t=56 d) + 15 (t=60 d)	6	freeze-dried *Chlorella*	97	*(87-)* **97**	68	2.6	
B-Chlo8	26 = 5(t=0) + 6 (t=56 d) + 15 (t=60 d)	8	freeze-dried *Chlorella*	97	*(46-)* **53**	21	0.8	30
B-Chlo10	26 = 5(t=0) + 6 (t=56 d) + 15 (t=60 d)	10	freeze-dried *Chlorella*	97	*(5-)* **15**	67	2.6	
B-Chlo12	26 = 5(t=0) + 6 (t=56 d) + 15 (t=60 d)	12	freeze-dried *Chlorella*	97	*(35-)* **45**	79	3.0	40
C-Phaeo10	50	10	fresh *Pheaodactylum*	50	*(1-)* **11**	669	13.4	193
D-Phaeo8	28	8	fresh *Pheaodactylum*	87	*(11-)* **15**	400	14.3	90 - 80 - 150
D-Phaeo10	28	10	fresh *Pheaodactylum*	87	*(11-)* **15**	606	21.6	100 - 90
D-Phaeo12	28	12	fresh *Pheaodactylum*	87	*(8-)* **11**	580	20.7	140 - 80 - 150
D-Phaeo14	28	14	fresh *Pheaodactylum*	87	(8-) **11**	450	16.1	100 - 90 - 50

Table 3.3: Quantitative results of the reproduction experiments with B. marginata.

3.1.4.1. Influence of different temperatures on the reproduction of *B. marginata*

The results of the different experiments suggest that temperature has an influence on the delay before the first reproduction (Figure 3.2, Table 3.3) and on the amount of produced juveniles (Figure 3.3, Table 3.3).

For experiment A (fed with *Chlorella*), the time before reproduction is 68 days at 6°C against 34 days at 8, 10 and 14°C (observations every 3 to 10 days) (Figure 3.2). Also in experiment B, at 6°C the time before reproduction is much longer (97 days) compared to the culture jars at higher temperatures; a minimum time is observed for the jar at 10°C (15 days). Only in experiment D, the difference between the jars is very small, and at all temperatures, reproduction happened within 15 days. Considering the number of juveniles produced per adult added in the experiments (Figure 3.3, Table 3.3), maximum numbers are generally observed at intermediate temperatures. For example, in experiment A (fed with *Chlorella*), $R_{juveniles/adults}$ is about 10 at 8°C and 10°C, but

only about 6.5 at 6°C and 14°C (Table 3.3). The same tendency can be observed in experiment D (fed with *Phaeodactylum*) where about 21 juveniles are produced per adult added at 10°C and 12°C compared to 14.3 and 16.1 juveniles per adult at 8°C and 14°C, respectively (Figure 3.3, Table 3.3). Only in experiment B (with *Chlorella*), a minimum $R_{juveniles/adults}$ (0.8) is observed at 8°C (B-Chlo8). At the three other temperatures (6, 10 and 12°C), we observed about 3 juveniles produced per adult.

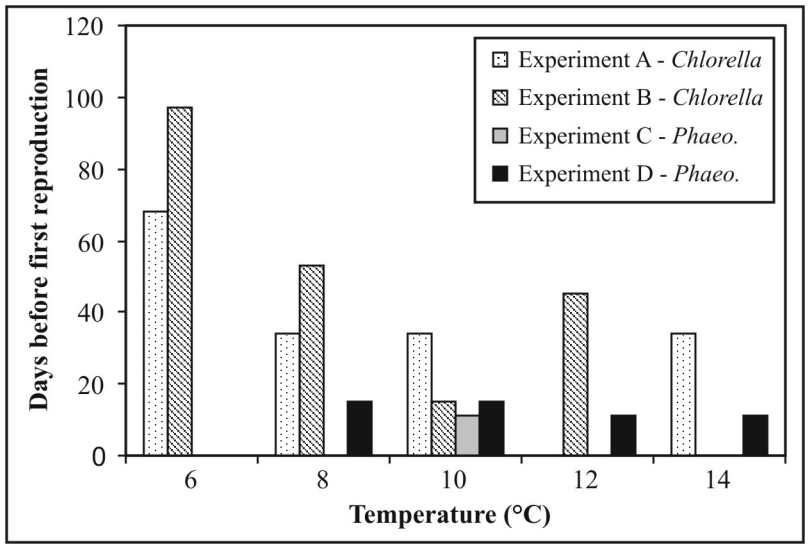


Figure 3.2: Time before the first reproduction of B. marginata *occurs in experiments A, B* (Chlorella), *C and D* (Phaeodactylum).

3.1.4.2. Influence of different food particles on the reproduction of *B. marginata*

The time before the first reproduction is longer in cultures fed with *Chlorella* than in those fed with *Phaeodactylum* (48 versus 13 days on average, respectively; Table 3.3). This time is about similar (11 and 15 days, respectively, with no observations in between) for C-Phaeo10 and D-Phaeo10, both with *Phaeodactylum* at 10°C. The time varies between 15 and 97 days for experiments with *Chlorella*. With the exception of experiment B-Chlo10 (15 days), the time before the first reproduction is always much longer in cultures fed with freeze-dried *Chlorella* than in those fed with *Phaeodactylum*.

The quantity of juveniles obtained in experiment D, fed with *Phaeodactylum* (509 juveniles on average) is higher than that obtained in experiment A, fed with *Chlorella* (202 specimens on average) (Figure 3.3, Table 3.3). The numbers of juveniles obtained in experiment B (fed with

Chlorella) are extremely low (between 21 and 79 juveniles) compared to the other experiments (number of juveniles always above 150). A maximum number of 669 juveniles is observed in experiment C-Phaeo10 (10°C). However, in this experiment 50 adults where introduced at the beginning, about 2 times more than in all other experiments. The $R_{juveniles/adults}$ ratio confirms the differences in the juvenile production between the types of food particles: the number of juveniles produced per adult added in the experiment is higher with *Phaeodactylum* (between 13.4 and 21.6 juveniles per adult) than with *Chlorella* (between 0.8 and 9.8 juveniles per adult).

Also the number of juveniles produced per reproduction (when counting was possible) is different according to the diet. On average, 30 juveniles were released per reproduction event in cultures fed with *Chlorella*, against 110 juveniles in cultures fed with *Phaeodactylum* (Table 3.3).

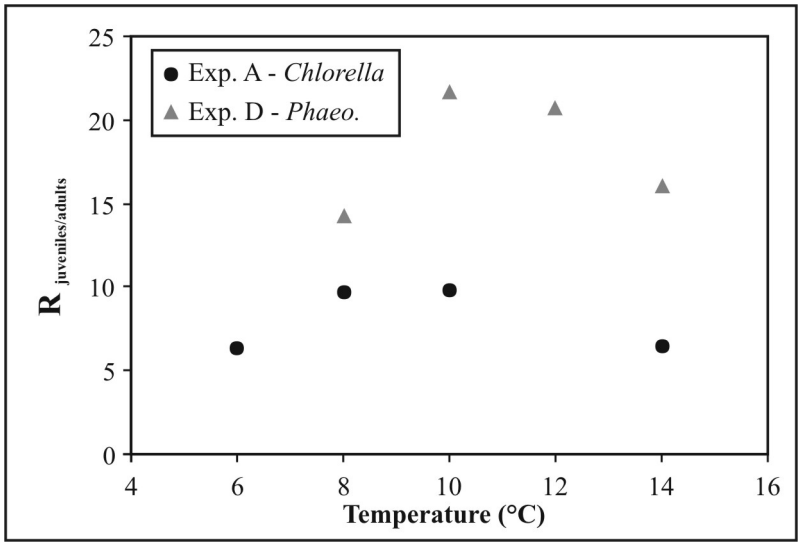


Figure 3.3: Number of juveniles produced per adult of B. marginata *added in the culture jars ($R_{juveniles/adults}$) in experiments A* (Chlorella) *and D* (Phaeodactylum). *Results for experiment B are not presented since this experiment was ended too soon after the introduction of the majority of the adult specimens (see § 3.1.5 for explanation).*

3.1.5. Analysis of possible problems in our reproduction experiments

In all cases, except for experiment A, adult specimens were fed with less than 2 mg of *Chlorella* for a maximum of 2 days before the onset of the experiment in order to distinguish living specimens. The fact that this 2-days feeding period before the onset of the experiment was not applied in experiment A can not explain the much longer (than 2 days) delay in reproduction compared to experiments C and D.

The number of juveniles obtained in experiment B is systematically very low. However, experiment B is particular, since only 5 adults were added per crystallising jar at the beginning of the experiment. The other adults (21 individuals) were added 56 and 60 days after the beginning of the experiment (Table 3.3). In experiment A, where foraminifera were also fed with freeze-dried *Chlorella*, the average time to obtain the first reproduction was 42.5 days (standard deviation 17 days) (Table 3.3). It is possible that part of the specimens added later during experiment B (56 and 60 days after the beginning) did not have the time to reproduce, since the experiment was ended about 40 days after their introduction. This may explain why much less juveniles were produced in experiment B than in experiment A. Therefore, the data of experiment B can not be compared directly with those of the other experiments.

In the 6°C setup of experiment B, reproduction happened just before the end of the experiment and the juveniles (68 specimens) were still single-chambered and uncalcified when the experiment was ended. It is possible that part of these juveniles would not have calcified their first chamber if the experiment had continued. Such a phenomenon was observed in a reproduction event of experiment B-Chlo8 (*Chlorella* at 8°C) where 30 juveniles were produced, whereas only 21 juveniles with a calcified test were found at the end of the experiment. Between the reproduction and the end of the experiment, 9 juveniles disappeared, most probably because they did not succeed to calcify their first chamber. For this reason, the number of juveniles obtained at 6°C is not comparable to the number of juveniles counted in the other culture jars of experiment B, and therefore we will not further consider this experiment.

3.2. Growth experiments

Table 3.4 summaries the culture conditions and main results of the test growth experiments (I to VI). The percentage of juveniles that calcified at least one new chamber in the experiments is indicated (14 to 96%). For all growth experiments (I to VI), the added juveniles were marked with calcein (Plate 3.1, Fig. A-1) so that it was possible at the end of the experiment to distinguish between the specimens that had calcified new chambers (Plate 3.1, Figs. A-2 and 3) and the specimens that did not calcify. Several possibilities may explain why some specimens did not add new chambers in the culture jars: (1) they may have died before their addition to the experiment (they died in the calcein bath after they calcified their first chambers), (2) they may have died during the experiment, or (3) they were alive but did not calcify a new chamber. Within one experiment, the results of the various culture jars can always be directly compared, since the

applied protocol was the same for all treatments. We calculated a test fragility rate, on the basis of the percentage of broken juvenile specimens compared to the number of juvenile specimens which had calcified one or more new chambers at the end of the experiment (1 to 48%, Table 3.4-a). Either these specimens broke in the experiment jar, or they were so fragile that they broke when they were picked out of the experiment with a fine brush. Table 3.4-a also specifies the mean test growth rates and standard deviations (calculation based on the foraminiferal length) obtained in each experiment as well as the coefficient of biovolume growth ($R_{biovolume}$, see § 2.3 for explanation). All the average data of length, width and biovolume for each experiment are presented in Appendix 3.1. For experiment VI, which aims to test the influence of different temperatures on chamber addition, test growth rates were not calculated. Since these experiments last only 20 days, the increase in foraminiferal length during the experiments was not sufficiently high to allow us to perform reliable calculations. In this experiment, we counted for each specimen the number of new chambers calcified in the culture jars (Table 3.4-b).

(a)

Culture jars	Temperature (°C)	Food type	State of the food	Experiment duration (days)	% of newly calcified juveniles	Fragility rate (%)	Mean test growth rate based on length (% per month)	Standard deviation (% per month)	$R_{biovolume}$
I-C_1	10	*Chlorella*	freeze-dried	31	76	26	57	28	3.0
I-C_2		*Chlorella*	freeze-dried	31	79	38	55	28	3.0
I-C_3		*Chlorella*	freeze-dried	31	34	48	17	16	1.5
II-C	10	*Chlorella*	freeze-dried	28	87	32	49	29	2.7
II-C+P		*Chlorella* + *Phaeodactylum*	freeze-dried + fresh	28	87	7	105	31	5.9
II-P		*Phaeodactylum*	fresh	28	81	8	151	40	10.1
III-C	10	*Chlorella*	freeze-dried	29	94	34	120	42	7.4
III-C+P		*Chlorella* + *Phaeodactylum*	freeze-dried + fresh	29	91	29	134	49	8.7
III-P		*Phaeodactylum*	fresh	29	87	9	128	40	7.9
IV-P	10	*Phaeodactylum*	fresh	34	75	3	60	37	3.4
IV-A		*Amphiprora*	fresh	34	86	21	115	54	7.3
V-D_1	10	*Dunaliella*	fresh	35	89	1	55	24	3.1
V-D_2		*Dunaliella*	frozen	35	90	19	46	24	2.6
V-P_1		*Phaeodactylum*	fresh	35	91	21	80	34	4.7
V-P_2		*Phaeodactylum*	frozen	35	96	16	66	23	3.6

(b)

Culture jars	Temperature (°C)	Food type	State of the food	Experiment duration (days)	% of newly calcified juveniles	No calcification (nb of sp.)	1 new chamber calcified (nb of sp.)	2 new chambers calcified (nb of sp.)	3 new chambers calcified (nb of sp.)	More than 3 new chambers calcified (nb of sp.)
VI-A8	8	*Amphiprora*	fresh	20	14	83	13	1		
VI-A10	10			20	42	54	36	3		
VI-A12	12			20	75	24	23	39	7	2
VI-A14	14			20	64	37	23	31	10	1

Table 3.4: Results of the growth experiments with B. marginata*: (a) for experiments I to V where the mean test growth rates were calculated on the basis of foraminiferal length and where $R_{biovolume}$ corresponds to the biovolume growth coefficient, and (b) for experiment VI where the number of newly formed chambers has been counted for each individual.*

3.2.1. Experiments with different diets

3.2.1.1. Percentage of newly calcified juveniles and test growth rates

With the exception of the culture jar I-C_3 (8 mg of freeze-dried *Chlorella),* for all the food experiments (I to V) the percentage of juveniles that calcified new chambers within one month was always at least 75% (Table 3.4-a).

Figure 3.4 shows the increases of test size for the juveniles of *Bulimina* for experiments I to IV (Figure 3.4-a) and V (Figure 3.4-b). These results are presented separately since the starting pools of juveniles are different in size and foraminiferal growth is not uniform (Bradshaw, 1957; Stouff *et al*., 1999a). In experiment I, with different quantities of *Chlorella* (jars I-C_1 to I-C_3), the test growth rates of the specimens cultured with 2 and 4 mg of *Chlorella* (I-C_1 and I-C_2) were not significantly different (Df=111, t=0.22, p<0.001). On the average, these two treatments show a mean test size increase of 56% per month, which roughly corresponds to a threefold increase in biovolume. In the treatment with 8 mg of *Chlorella* added (I-C3), the average test growth rate is only 17% per month (corresponding to a 1.5 increase in biovolume). Since in this treatment only 34% of the population added one or more chambers, it appears that the conditions were less favourable, which could explain the lower test growth rate.

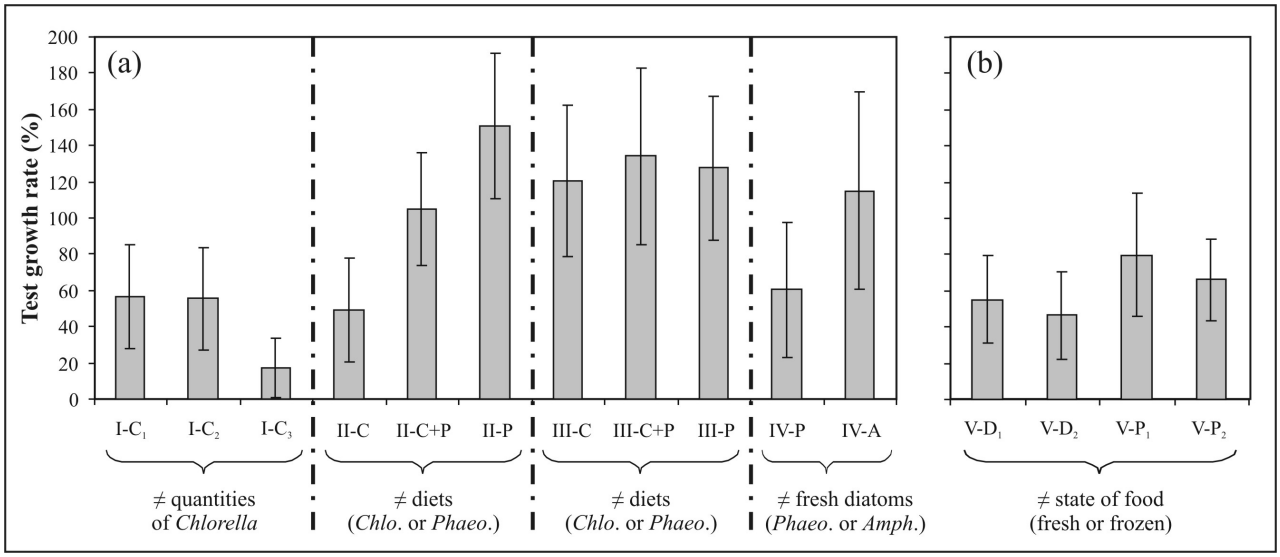


Figure 3.4: Test growth rates of the juveniles measured in experiments I to V, testing the influence of the food. The average size of the pool of juveniles at the beginning of the experiments was (a) 93 µm for experiments I, II, III and IV, and (b) 158 µm for experiment V.

For the three treatments of experiment II, comparing the influence of different food types, test growth rates of foraminifera are significantly different (*Df*=106-104-102, *t*=9.66-6.61-14.90, *p*<0.001). The population of calcifying specimens showed a size increase of about 150% in only one month when fed with only *Phaeodactylum* (II-P), whereas the size increase was only about 50% per month in treatment II-C, where foraminifera were only fed with freeze-dried *Chlorella*. The experiment with a mix of the two types of food (II-C+P) yielded an intermediate value with a test growth rate of about 100% per month (Figure 3.4-a, Table 3.4-a).

Experiments I-C_1 and II-C can be considered as exact replicates since the feeding conditions were rigorously the same (2 mg of *Chlorella*) and the pools of added juveniles had the same size at the beginning of the experiment. There is no statistically significant difference between the test growth rates, with values of 57 and 49% per month, respectively (*Df*=108, *t*=1.35, *p*<0.001). Also the percentages of actively calcifying juveniles are of the same order of magnitude with 76 and 87%, respectively for I-C_1 and II-C.

The three treatments of experiment III present the same food conditions (three different food types) as experiment II, except that in experiment III, food was added twice: once at the beginning and once 15 days after the start of the experiment. No additional food was given for experiment II after the start of the experiment. The results obtained for experiments III are significantly different from those of experiment II. For the two treatments fed with only *Chlorella*, juveniles present a test growth rate of 120% per month for III-C (two food additions) compared to only 49% per month for II-C (a single food addition; Figure 3.4-a) (*Df*=113, *t*=10.78, *p*<0.001). These average test size increases correspond to estimated foraminiferal biovolume increases with factors of 7.4 and 2.7 for III-C and II-C, respectively. These data suggest that test growth is faster when *Chlorella* is added twice. In the two treatments where foraminifera are fed with a mixture of *Chlorella* and *Phaeodactylum*, the differences in test growth rate are smaller, but still siginificant: 134% test size increase per month for III-C+P (2 food additions) against 105% for II-C+P (single food addition) (*Df*=115, *t*=3.95, *p*<0.001). Differences in test growth rate are no longer significant in treatments where foraminifera are only fed with *Phaeodactylum:* 128% test size increase for III-P (2 food additions) versus 151% per month for II-P (a single food addition) (*Df*=117, *t*=3.14, *p*<0.001). Rather surprisingly, in experiment III, where food has been added twice, the test growth rates are no longer statistically different between the three tested food types (*Df*=122-128-128, *t*=1.68-0.82-1.01, *p*<0.001). The average test growth rate for the juvenile specimens, of 127% per month, corresponds to an estimated 8-fold increase of biovolume.

Experiment IV was designed to compare the influence of two different species of fresh diatoms: *Phaeodactylum* (IV-P) and *Amphiprora* (IV-A). The specimens fed with *Amphiprora* (IV-A) showed a higher test growth rate (115% per month) than those fed with *Phaeodactylum* (IV-P, 60% per month) (Df=118, t=6.50, p<0.001; Figure 3.4-a, Table 3.4-a).

Experiment V was performed to test the impact of the state of the food on the foraminiferal test growth. Either *Dunaliella* or *Phaeodactylum* was added fresh, directly pipeted from the food culture medium, or frozen. The number of juveniles that calcified new chambers was high in all cases (more than 89%; Table 3.4-a) and was very similar irrespective of the state of the food (fresh or frozen) and the type of food (green algae or diatoms). The average length of the specimens increased by about 100 µm (Appendix 3.1). Test growth rates are significantly higher for the two treatments with *Phaeodactylum* (80% per month for V-P_1 and 66% per month for V-P_2; biovolumes multiplied by 4.7 and 3.6, respectively) than for the two treatments with *Dunaliella* (55% per day for V-D_1 and 46% per day for V-D_2; biovolumes multiplied by 3.1 and 2.6, respectively) (Figure 3.4-b and Table 3.4-a). No significant difference of test growth was found between the two states of the food (fresh or frozen), neither for *Phaeodactylum* (Df=142, t=2.22, p<0.001), nor for *Dunaliella* (Df=150, t=2.95, p<0.001).

3.2.1.2. Fragility and abnormalities of foraminiferal tests

The fragility rate presented in Table 3.4-a corresponds to the percentage of broken specimens within the total number of specimens that had calcified at the end of the experiments. The fragility rate ranges from 1 to 48%. It tends to be higher in the experiments with only *Chlorella* (I-C_1, I-C_2, I-C_3 with 26, 38 and 48% respectively, II-C with 32% and III-C with 34%, versus an average of 13% for all other experiments). Breakage of the fragile foraminifera occurred always in the new calcite formed during the experiment. It appears that feeding with freeze-dried *Chlorella* has an adverse effect on the quality of the newly formed calcite. The pH was measured using a macroelectrode at the end of experimental treatments I-C_1, I-C_2 and I-C_3 and showed a pH of 8.2 in the overlying waters. Therefore, the fragility of the foraminiferal tests seems not to be the consequence of a drop in pH. However, it can not be excluded that the pH in the microenvironment within the thin layer of *Chlorella* deposited on the bottom of the jar was lower, and was not detected with our macroelectrode measurements.

In experiment II, juveniles fed only with *Chlorella* (treatment II-C) presented a less typical chamber form, and had a much lower test growth rate than specimens from the other two treatments (II-C+P and II-P; Plate 3.1, Figs. C-1, 2 and 3).

Generally, juveniles that calcified in the treatments fed with *Chlorella* presented less sharply angled undercuttings at the base of each chamber (Plate 3.1, Fig. C-1), and the calcite had an irregular surface aspect. The only exception was the juveniles from experiment III-C, which presented almost no morphological abnormalities. In this treatment, the test size increase was larger than in all other treatments fed with *Chlorella,* so that these juveniles could attain a more advanced ontogenetic stage with the sharp undercuttings typical of *Bulimina marginata*.
In the experiments where *Chlorella* was mixed with another food (fresh *Phaeodactylum*), the fragility rates varied from 7 to 29%, without showing a clear tendency.

3.2.2. Experiments with different temperatures

Because *Amphiprora* was easy to culture in the laboratory, and the foraminiferal test growth rate was satisfactory with these diatoms, we decided to test the influence of different temperatures (8, 10, 12 and 14°C) on foraminifera fed with this food type. After 20 days, the percentage of juveniles that showed tests growth in the experiments points out a clear correlation with temperature. At 12°C (VI-A12) and 14°C (VI-A14), a large majority of the juveniles calcified new chambers (75 and 64% of the initial population, respectively). On the contrary, only 42% of the juveniles formed at least one new chamber at 10°C whereas at 8°C only 14% of the population calcified one or two new chambers (Table 3.4).
After the first 11 days of experimentation, some specimens had already calcified new chambers at 12 and 14°C whereas specimens started to add new chambers only after 14 days at 10°C. At 8°C, newly formed calcite was only observed at the end of the experiment, after 20 days.

To estimate the test growth of the juveniles used in this experiment, specimens were not measured. In 20 days, juveniles did not have the time to calcify enough to notice a measurable difference in size between their length at the beginning and at the end of the experiment. Therefore the number of newly formed chambers was counted for each individual. Figure 3.5 and Table 3.4-b present the number of specimens without chamber addition, as well as the number of specimens that calcified 1, 2, 3 or more new chambers according to the four tested temperatures. Only very few specimens

from the treatments at 8°C (VI-A8) and 10°C (VI-A10) calcified more than 1 new chamber in the culture jars, whereas 48 and 42 specimens calcified 2 chambers or more in the treatments at 12°C (VI-12) and 14°C (VI-14), respectively. At 8°C, all the specimens showing test growth calcified only 1 new chamber in 20 days except 1 specimen that calcified 2 chambers. At 10°C, the number of specimens that added a new chamber is 3 times higher than at 8°C (VI-A8) but only 3 specimens calcified more than 1 chamber. At higher temperatures, the relative proportion of foraminifera that calcified is higher and the calcifying individuals added more chambers. Test growth appears therefore to be strongly influenced by temperature.

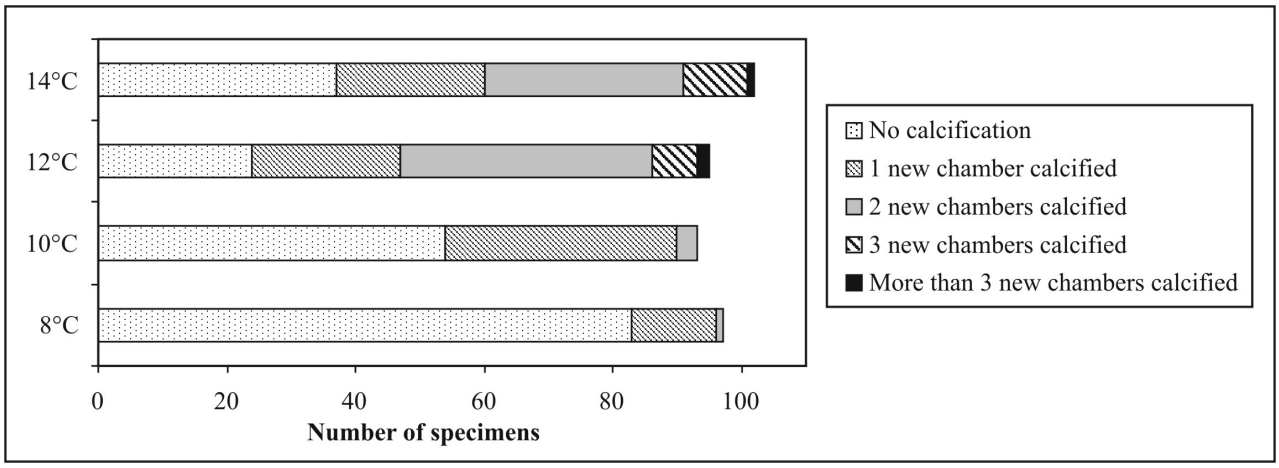


Figure 3.5: Experiment VI: Test growth of the juveniles of B. marginata *at different temperatures (8, 10, 12 and 14°C).*

4. DISCUSSION

4.1. Reproduction of Bulimina marginata

The four experiments (A-D) aiming to optimise reproduction of *Bulimina marginata* in laboratory experimental conditions have allowed us to develop methods (1) to distinguish between living and dead individuals, and (2) to stimulate the reproduction of *Bulimina marginata* in a few weeks after incubation. The most practical way to select living individuals was to isolate them, and offer them a sufficient quantity of algal food. Living specimens ingested the food particles and their cytoplasm obtained the same colour as the ingested algae. Previously, Murray (1963) described this phenomenon for cultures of *Elphidium crispum*. Dead specimens, which did not ingest algae, did not show such a colour change. Food ingestion appears to stimulate test growth of juveniles,

but also reproduction of adult specimens. It is possible that the response to food input is particularly strong after keeping the foraminifera for a long time in sediment without adding fresh food. In fact, Bradshaw (1961) observed neither reproduction nor growth in cultures without sediment fed with a very low food concentration.

As was described previously by Murray (1963) and Heinz *et al*. (2005), after food addition, the food particles are rapidly accumulated by the living adults, which form a cyst. In our experiments, these cysts contained uniquely the food particles gathered around the foraminifer since no sediment was added in the culture jars. It is probable, that by building a cyst, foraminifera create a microenvironment around them. Different types of cysts have been distinguished according to their function: feeding cysts, reproduction cysts, growth cysts and protective cysts (see review in Heinz *et al*., 2005). In our experiments, specimens with two types of cysts were regularly observed with their aperture, as well as the cyst, against the bottom of the culture jar.

The first type of cyst was formed around living adults for which the food particles were concentrated around the aperture and did not cover the entire shell; these specimens were not ready to reproduce. These cysts are difficult to ascribe to a particular function although they seem to be used as a food stock. Specimens disposing of such feeding cysts did not always show formation of new chambers. In the second type of cyst, food particles were covering the entire test of the adult specimen; this appeared mainly to happen with specimens ready to reproduce. These reproduction cysts can easily be recognised for *Bulimina marginata.* They were previously observed in culture experiments for other foraminiferal taxa such as *Trochammina* spp. (Angell, 1990; Pawlowski *et al*., 1995), *Ammonia tepida* (Stouff *et al*., 1999a) and *Adercotryma glomerata* (Heinz *et al*., 2005). Angell (1990) and Pawlowski *et al*. (1995) reported that in *Trochammina* spp., after formation of the reproduction cyst, the entire cytoplasm of the parent individual leaves the (undamaged) test, and fills the cyst. Thereafter, the cytoplasmic division occurs inside the reproduction cyst. Such a phenomenon has never been observed in our experiments. However, since we could not make observations within the non-transparent reproduction cysts, we can not exclude that a similar mechanism occurs during the reproduction of *B. marginata*. Also in our experiments, part of the empty shells of the parental adult individuals was found intact, without traces of dissolution or broken chambers.

In our experiments we only observed asexual reproduction, or schizogony (multiple fission); systematically a single parent individual was found in the reproduction cyst and, after the

cytoplasmic division, the empty test stayed in the middle of the offspring (Figure 3.1 and Plate 3.1, Fig. B). Consequently, all produced juveniles were megalospheric. In culture experiments, asexual reproduction appears to be by far the most common way to reproduce for foraminifera (Bradshaw, 1957; Angell, 1990; Pawlowski *et al*., 1995; Stouff *et al*., 1999a, 1999b, 1999c; Nigam and Caron, 2000; Le Cadre and Debenay, 2006). This dominance of asexual reproduction is also found in nature (review in Lee *et al*., 1991), but may be reinforced by the artificial experimental conditions. However, sexual reproductions of *Ammonia tepida* have been noticed in culture experiments by Goldstein and Moodley (1993) and Stouff *et al*. (1999a) by the observation of biflagellated gametes. In our experiments, all parental specimens died shortly after the division of their cytoplasm and the release of the juveniles. This phenomenon is generally accepted (review in Lee *et al*., 1991). On rare occasions, Stouff *et al*. (1999a) and Le Cadre and Debenay (2006) observed that the adult specimens of *Ammonia tepida* could survive, when residual cytoplasm stayed inside the test. Such specimens have never been observed to reproduce again and they presented often deformations of the newly calcified chambers.

We noticed in our experiments that shortly after an asexual reproduction event, the juveniles released into the surrounding environment were only composed of a cytoplasm volume, without calcitic shell (Figure 3.1-A). This stage has earlier been observed by Bradshaw (1957), who described these early juveniles as small *Allogromia*-like forms, and has been illustrated by Stouff *et al*. (1999a). In our experiments, the proloculus (first chamber) was calcified 1 to 2 days after the release of the uncalcified juvenile specimens. During these first two days, part of the juveniles does not succeed to calcify the first chamber. These individuals probably die, and their cytoplasm rapidly disappeared. Once they are released from the reproduction cyst, the juveniles create their own protective and/or feeding cyst, by surrounding themselves with a thin layer of food particles. The initial parental reproduction cyst started to progressively disperse when the juveniles attained a 2 to 3 chambers stage (after 3 to 4 days). Angell (1990) noticed that juveniles of *Trochammina inflata* all left the reproduction cyst after the third chamber was completed.

Growth of juveniles of *Bulimina marginata* from a stage with only a calcified proloculus to a stage with 2-3 chambers is relatively fast (3 to 4 days). After this initial stage, the rate of chamber addition slows down. Earlier, Bradshaw (1957) remarked that test growth in *Ammonia beccarii tepida* is not uniform. For this reason, the size of the starting pool of juveniles is important in the growth experiments performed in this paper. Stouff *et al*. (1999a) observed that, in cultured

specimens of *Ammonia tepida,* the third chamber was formed within 24 hours after the release of the already calcified juveniles from the reproduction cyst, and the fourth chamber was formed in a few hours. This suggests that calcification is slower in our deep sea taxon *B. marginata* than in the coastal species *A. tepida*.

4.2. Influence of temperature on foraminiferal reproduction and growth

After our experiments, we can be sure that *Bulimina marginata* is able to reproduce, grow, and calcify new chambers at all tested temperatures between 6 and 14°C. In the natural environment, typical *marginata* morphotype like most of the specimens incubated in our experiments is found at water depths from 10 to 2200 m (Barmawidjaja *et al*., 1992; Jorissen *et al*., 1998; De Rijk *et al*., 2000; Fontanier *et al*., 2002; Mendes *et al*., 2004; Bergin *et al*., 2006; Eberwein and Mackensen, 2006; Langezaal *et al*., 2006; Mojtahid *et al*., 2006, 2008; Szarek *et al*., 2006; Di Leonardo *et al*., 2007; Koho *et al*., 2007; Abu-Zied *et al*., 2008; Brückner and Mackensen, 2008; Panieri and Sen Gupta, 2008; Eichler *et al*., in press; Frezza and Carboni, in press; Pascual *et al*., in press), corresponding to a temperature range from approximately 3 to 20°C. Additional experiments we performed in the laboratory (not presented here) show that *Bulimina marginata* is able to form new chambers and reproduce at temperatures from 4 to 19°C.

In our experiments with freeze-dried *Chlorella* (A and B), temperature appears to influence the time before the occurrence of the first reproductive event. In both experiments, reproduction appeared much later at 6°C (after 68 and 97 days, respectively), than in the experiments performed at 8, 10, 12 and 14°C (between 15 and 53 days, without a further correlation with temperature at higher temperatures, Figure 3.2). In the experiments with fresh *Phaeodactylum*, temperature does not appear to effect the time until the first reproduction event.

Temperature also appears to influence the rate of chamber addition of the juveniles. In growth experiment VI, we observed that a larger percentage of the individuals added new chambers at 12 and 14°C (75 and 64%, respectively) than at 10°C (42%) (Figure 3.5). At 8°C, only a minor part (14%) of the specimens calcified most times a single new chamber. There is also a very clear tendency of more chambers added at higher temperatures (Figure 3.5).

The results of reproduction experiments A and D also tend to suggest that more juveniles are produced in total at intermediate temperatures than at 6°C or at 14°C (Figure 3.3), both in the cultures fed with *Chlorella* (maximum at 8-10°C) and with *Phaeodactylum* (maximum at 10-

12°C). Considering that the quantity of juveniles produced per reproduction event is apparently not depending on the temperature (Table 3.3), these results would imply that more adults succeeded to reproduce at intermediate temperatures. However, these data are not sufficiently different to clearly recognise a consistent tendency. Further experiments are necessary to confirm these observations. Unfortunately, these data can not directly be compared with those of experiment B, since that experiment was ended too soon after the introduction of the majority of the adult specimens.

Earlier, Bradshaw (1955, 1957, 1961) studied the influence of temperature, salinity and food on the test growth rate and reproductive activity of shallow benthic foraminifera. The author reported a lower growth rate for *Ammonia beccarii tepida* at lower temperatures: a minimum at 15°C (4 µm/10 days) and a maximum at 30°C (84 µm/10 days). Beyond an upper threshold limit (32-35°C), growth ceased entirely. Also at 10°C, no test growth was observed but the individuals survived for a long time, without reproducing or adding new chambers (Bradshaw, 1957). As soon as these specimens were re-introduced in a culture jar with a convenient temperature (24-27°C), they added chambers and reproduced rapidly. In our growth experiment at 8°C (experiment VI), we observed that a very low proportion of the population calcified new chambers. Also in our experiment, the specimens that did not add chambers were not dead but non-active, probably as a result of the unfavourable temperature conditions.

The effect of temperature on the metabolism of marine organisms is well known, e.g. in fishes (Strawn, 1961; Wheaton, 1977; McCullough, 1999; Person-Le Ruyet *et al*., 2004), mussels larvae (Sprung *et al*., 1984) or algae (Seaburg *et al*., 1981). Our observations appear to show that temperature has an impact on the behaviour of deep-sea benthic foraminifera as it was also observed for shallow benthic foraminifera by Bradshaw (1961). Previously, Bijma *et al*. (1990) demonstrated a clear temperature effect on the calcification of different species of planktonic foraminifera. To our knowledge, this is the first time that such a temperature effect is shown by laboratory experiments for deep-sea benthic foraminifera.

We think that the different behaviour of our foraminiferal cultures at different temperatures is not due to different periods of adaptation of foraminifera which originally lived at another temperature (i.e. 10°C). If this should be the case, individuals introduced at a higher temperature should show a similar delay before reproduction as those introduced at lower temperature. Since this is not at all the case, we think that these delays are due to a slowing down of physiological processes at lower

temperatures. Generally, individuals of a species will survive in a relatively large temperature range, grow in a smaller range, and reproduce in an even narrower range. Our results, with reproduction and calcification occurring at all tested temperatures, from 6 to 14°C, suggest that *Bulimina marginata* is a eurythermal species able to live and calcify at an even wider range of temperatures than the one tested here.

4.3. Influence of the diet on foraminiferal reproduction and growth

4.3.1. Different quantities of food

In growth experiment I, we investigated the eventual influence of different quantities of *Chlorella* (2, 4 and 8 mg per culture jar of 60 ml, respectively) on the test growth of juveniles of *Bulimina marginata*. In all three treatments, juveniles added new chambers, but in the treatment with 8 mg of *Chlorella* this concerned only one third of the specimens, whereas in the other two treatments, with lower quantities of *Chlorella*, more than 75% of the juveniles calcified one or more new chambers. The specimens fed with 8 mg of *Chlorella* showed also a lower increase of test size, than those fed with smaller quantities. As was suggested by Bradshaw (1955, 1961), it is possible that an excess of food may cause unfavourable conditions for foraminifera because of the high bacterial concentrations that occur when the food particles decompose. The bacterial activity could create low pH conditions in the thin layer of *Chlorella* settled at the bottom of the crystallising jar. It is possible that this putatively more acidic microenvironment around the foraminifera has not been detected by our macroelectrode measurements of pH in the overlying water. Such a decrease of pH in the environment surrounding the foraminifera could also explain the high percentage of fragile tests observed in many of the experiments.

The average observed test growth rates are about similar for the treatments with 2 and 4 mg of *Chlorella*, and about two times higher than those observed in the 8 mg treatment. Earlier, also Bradshaw (1961) observed a different behaviour in shallow foraminifera (*Ammonia beccarii tepida*) fed with different quantities of *Dunaliella* (heated at 50°C). Test growth rate increased when larger amounts of food were offered and no growth was observed below a certain concentration (112 cells/mm²). Apparently, in our experiments, we were always well above such a lower limit.

4.3.2. Different food types

In our experiments, a difference in reproductive activity is observed according to the type of food: freeze-dried *Chlorella* or fresh *Phaeodactylum*. The time before the first reproduction is much longer in experiments with *Chlorella* (48 days on average for experiments A and B) than in experiments with *Phaeodactylum* (13 days on average for experiments C and D). Such periods are of the same order of magnitude as the periods of 15 to 30 days observed for *Ammonia tepida* by Le Cadre and Debenay (2006) in their cultures fed with fresh diatoms.

The total number of juveniles picked at the end of the experiments (and also the number of juveniles produced per adult added) is always much higher in experiments with *Phaeodactylum* (experiments C and D) than with *Chlorella* (experiments A and B) (Table 3.3). However, the data of experiment B can not be directly compared, because of the late introduction of a large number of the adults.

Also the number of juveniles produced per reproduction event is much higher in the presence of fresh *Phaeodactylum* (110 juveniles on average) than with freeze-dried *Chlorella* (30 juveniles on average). This difference in the number of offspring probably explains the higher total number of juveniles produced in experiments with *Phaeodactylum*. In fact, for an equal number of reproductions, the number of juveniles produced would be more than 3 times higher with fresh *Phaeodactylum* than with freeze-dried *Chlorella*.

Several laboratory experiments observed asexual reproductions of *Ammonia tepida,* with an offspring counting 28 to 32 individuals (Bradshaw, 1957), 12 to 24 individuals (Stouff *et al*., 1999a), or 11 to 18 individuals (Le Cadre and Debenay, 2006). Nigam and Caron (2000) and Pawlowski *et al*. (1995) also observed asexual reproductions of shallow benthic foraminifera and counted respectively 39 juveniles for *Rosalina leei* and 40 to 60 juveniles for *Trochammina* sp.. Heinz *et al*. (2005) found 54 juveniles inside and in the vicinity of the reproductive cyst formed by an agglutinated foraminifera (*Adercotryma glomerata*). Also the deep-sea benthic foraminifera *Uvigerina peregrina* reproduced in the laboratory experiments accomplished by Hintz *et al*. (2004), and produced up to 55 juveniles. The number of juveniles per reproduction event that we obtained in our experiments with *Chlorella* is of the same order of magnitude as these earlier observations (30 specimens), whereas the number of juveniles obtained in our experiments fed with fresh *Phaeodactylum* is significantly higher (110 specimens per reproduction event).

A different response of the foraminifera in function of these two types of food is also observed in the test growth experiments: higher growth rates are obtained with fresh *Phaeodactylum* than with freeze-dried *Chlorella*. The average biovolume of specimens grown with only fresh *Phaeodactylum* (II-P, III-P, IV-P and V-P_1) is multiplied by 6.5 in one month whereas with only freeze-dried *Chlorella* (I-C_1, I-C_2, I-C_3, II-C and III-C) there is only a 3.5 fold increase. The differential response to these two food sources may be due to a different biochemical composition of the food (green algae or diatoms) or to a different preservation (freeze-dried or fresh) of the food that may equally influence its quality.

4.3.3. Different state of the food particles

4.3.3.1. Freeze-dried food particles

Barbarro *et al*. (2001) studied the biochemical composition of microalgae (*Isochrysis galbana* clone T-ISO) to evaluate the influence of preservation techniques such as freeze-drying. Freeze-drying led to a significant loss in the carbohydrate content and in the relative percentage of polyunsaturated fatty acids (PUFAs). The PUFAs content decreased even further with increasing storage times. The decrease in carbohydrates and PUFAs values due to freeze-drying has also been reported by Cordero Esquivel *et al*. (1993) for two strains of diatoms, *Chaetoceros* sp. and *Phaeodactylum tricornutum*. There are two explanations for the loss of the high nutritional biochemical compounds: either the physical alteration of the cell wall due to the freeze-drying process itself (Barbarro *et al*., 2001), or the higher bacterial degradation of the organic matter facilitated by the freeze-drying treatment which tends to destroy cell walls (Cordero Esquivel *et al*., 1993).

PUFAs are thought to be essential for several physiological functions in marine organisms. They contribute significantly to mollusc bivalves diets (Barbarro *et al*., 2001) and Gulati and Demott (1997) demonstrated their importance in the reproduction efficiency in zooplankton.

The differential impact of fresh, frozen and freeze-drying food on the growth of *Ruditapes decussatus* (clams) was studied by Albentosa *et al*. (1997). They demonstrated that the growth of clam's larvae varied according to the process by which the microalgae (*Isochrysis galbana* clone T-ISO) were preserved. The highest weight was obtained for larvae fed with fresh food and the lowest for larvae fed with freeze-dried food. These authors observed similar acceptability (ingestion) but minimum digestibility values (absorption efficiency) when freeze-dried food was

offered, in comparison to fresh or even frozen food particles. For bivalves, several other studies have evidenced that the nutritional value of their food (common microalgae) is mainly determined by the PUFAs and carbohydrate contents (Enright *et al*., 1986; Whyte *et al*., 1990).
The importance of PUFAs for certain benthic foraminifera has been proved by Suhr *et al*. (2003). They measured significantly higher amounts of PUFAs in the calcareous species *Globocassidulina subglobosa* than in the phytodetrital food source surrounding the foraminifera. They concluded therefore that *G. subglobosa* feeds selectively on specific compounds of the organic matter. Since the freeze-dried *Chlorella* used in our experiments most likely is poor in PUFAs due to its preservation by freeze-drying, this could partly explain the less favaourable culture results with this food type in our experiments: the longer time before the first reproduction, the lower number of juveniles per reproduction event, and the lower test growth rates of the specimens. Freeze-dried *Chlorella* is well ingested by foraminifera since their cytoplasm become green after one day in contact with this diet. However, because of its lower nutritional value (less carbohydrates and PUFAs), foraminifera appear to dispose of a reduced energy budget, resulting in a slow-down of physiological processes such as reproduction and growth.

4.3.3.2. Mixtures of fresh and freeze-dried food particles

In experiment II, the addition of a small proportion of fresh microalgae to the freeze-dried food particles led to an increase in test growth rates; it was minimal with freeze-dried *Chlorella* (II-C), and maximal with fresh *Phaeodactylum* (II-P). The treatment with a mixture of these two food types yielded an intermediate test growth rate. Similar observations were made for example on clam larvae (Laing *et al*., 1990). These authors tested different quantities of fresh food in comparison to freeze-dried food, and investigated the proportion of freeze-dried food that could be added to obtain the same growth rates as with exclusively fresh diet. In our experiment II, the proportion of added freeze-dried food to fresh food was too high to attain a similar growth rate as with only fresh diatoms. However, in our experiment III, in which the three different food types were offered twice, the results become very comparable. Freeze-dried *Chlorella* contains less easily absorbed compounds (carbohydrates and PUFAs) due to the preservation process. It is possible that the second feeding results in the presence of labile food at a critical moment, for this reason the protocol with two feeding periods could yield better results with freeze-dried food.

4.3.3.3. Fragility of calcite and preservation processes

If we consider the percentages of broken specimens (fragility rate) in the different experiments fed with green algae and/or diatoms, we can see that juveniles fed with freeze-dried food construct more fragile tests than those fed with mixed or fresh food.

There is no obvious difference between the food type (green algae or diatoms). Figure 3.6 summaries the percentage of broken specimens, according to the food preservation, for all the experiments at 10°C (1) with **green algae** and (2) with **diatoms**. For both food types, we can see that the calcite formed in cultures with frozen or freeze-dried food is more fragile than the calcite formed in the presence of fresh food. As explained before, the fragility of the tests of specimens fed with freeze-dried food could result from a decrease of the pH in the microenvironment in which the foraminifera calcify, due to: (1) the higher concentration of food in experiments with freeze-dried food (e.g. 2 mg of dried particles) than in experiments with fresh food (e.g. 2 ml of particles diluted in the medium used in the algal cultures), and/or (2) to the high bacterial development in cultures with a freeze-dried diet (Urban and Langdon, 1984). It appears that bacterial activity is enhanced in the presence of dead food particles in which membranes are already partially destroyed due to preservation processes. This putative decrease in pH could lead to a disturbance of the carbonate system and provoke a perturbation of the calcification process. Another possibility to explain the calcification problems could be the impact of the preservation processes (freezing and freeze-drying) on the biochemical composition of the microalgae (loss in PUFAs and carbohydrates contents) (Babarro *et al*., 2001). The scarcity of easily digestible food could result in a lack of energy that may complicate the foraminiferal biomineralisation.

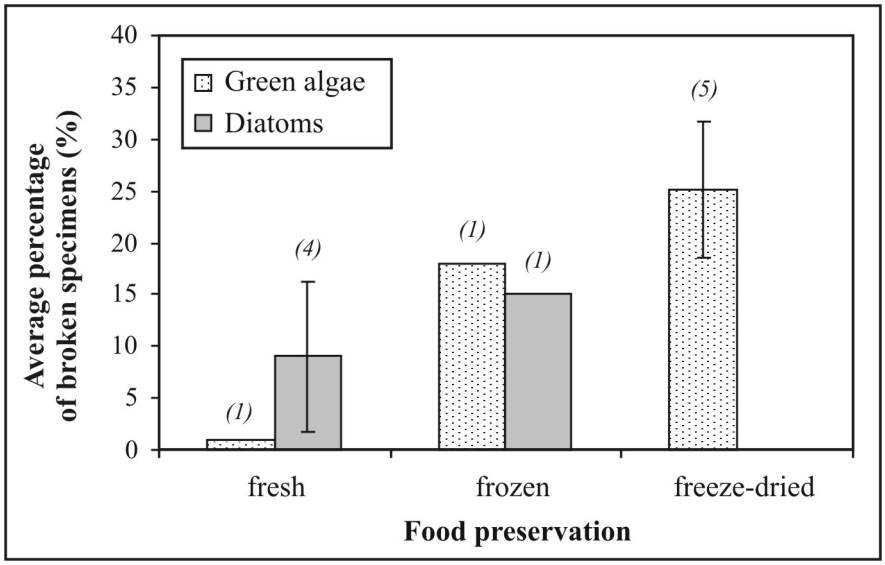


Figure 3.6: Percentage of broken specimens in all growth experiments at 10°C (1) with green algae: fresh Dunaliella *(V-D1, 1 culture jar), frozen* Dunaliella *(V-D2, 1 culture jar) and freeze-dried* Chlorella *(I-C1, I-C2, I-C3, II-C and III-C, 5 culture jars); and (2) with diatoms: fresh* Phaeodactylum *(II-P, III-P, IV-P and V-P1, 4 culture jars) and frozen* Phaeodactylum *(V-P2, 1 culture jar).*

4.3.4. Optimal experimental conditions for the reproduction and growth of B. marginata

The ultimate aim of the experiments described in this paper was to determine the ideal experimental conditions, which could lead to the rapid reproduction and test growth of deep-sea benthic foraminifera. Optimisation of these methods would largely increase the potential to obtain/verify calibration curves for paleoceanographical proxies (temperature, salinity, etc.) on the basis of laboratory experiments.

In this paper we investigated foraminiferal reproduction and test growth at different temperatures, and tried to determine the most suitable food, considering as well the nature of the offered particles, as their preservation. The extremely time-consuming character of the experiments made it in most cases impossible to have replicates. Nevertheless, some clear tendencies show up, which in our opinion can be used to optimise culturing of deep sea foraminifera.

It appears that *Bulimina marginata* reproduces and calcifies at all temperatures from 6°C to 14°C, and probably also at slightly lower and higher temperatures. There is a strong indication that much better results are obtained with fresh food particles than with frozen or freeze-dried food.

Furthermore, feeding with fresh diatoms leads to an accelerated reproduction, with a more abundant offspring. Finally, repeated feeding seems preferable when freeze-dried food is chosen, because it may provide labile food particles at critical intervals of the foraminiferal life cycle.

5. CONCLUSION

Several laboratory experiments, performed during two years, allowed us to observe in detail the asexual reproduction of the deep-sea foraminifer *Bulimina marginata*. Figure 3.1 summaries the different steps, and gives an indication of the length of each of the successive steps. Our experiments strongly suggest that temperature and food type may influence the reproduction and test growth of *Bulimina marginata*. Regarding the possible effect of temperature, the main results are:

- ✓ Reproduction and tests growth occur at all tested temperatures, from 6 to 14°C;
- ✓ The time before reproduction occurs is longer when the temperature is lower;
- ✓ It appears that more juveniles are produced at an intermediate temperature range (8-12°C, close to *in situ* temperature) than at lower or higher temperatures, whereas the quantity of juveniles produced per reproduction event seems to be independent of the temperature;
- ✓ The test growth rate, and also the increase of biovolume, are higher at higher temperatures;
- ✓ *Bulimina marginata* becomes less active at lower temperatures.

With respect to the effect of different food particles, the main results are:

- ✓ Reproduction and test growth occur both with green algae and with diatoms, irrespective of the preservation of the food particles;
- ✓ Feeding with fresh *Phaeodactylum* yields better results compared to feeding with freeze-dried *Chlorella*:
  - The time before reproduction is shorter,
  - A higher quantity of juveniles is produced, either in total and per reproduction event,
  - The test growth is faster, and the tests of the juveniles are less fragile.
- ✓ We think that the worse results obtained with *Chlorella* are due to the preservation by freeze-drying, which may decrease the carbohydrate and PUFAs contents of the food, thus decreasing the nutritious value of the algae;

- ✓ Rather surprisingly, offering freeze-dried *Chlorella* a second time during the experiment led to a considerable amelioration of the results, but did not solve the problem of the fragile tests of the individuals fed with this type of food.

It appears that *Bulimina marginata* can easily be cultured in the laboratory without sediment in a wide range of temperatures. The best results are obtained by adding fresh diatoms. Our improved protocol, defined after 2 years of experiments, allows us to perform experiments with deep sea foraminifera, aiming at a better calibration of paleoceanographical proxies based on the geochemical composition of the foraminiferal tests.

Acknowledgements

We would like to thank the crews of the Côtes de la Manche, our scientific ship during both sampling campaigns. We are grateful to the University of Tübingen that provided us the strain of algae *Dunaliella tertiolecta*. Thank you also to M. Gaultier for her technical assistance in the laboratory and to F. Lombard for his helpful comments on the manuscript.

Appendix 3.1 : *Additional quantitative results of the growth experiments I, II, III, IV and V with* B. marginata*. The average length, width and biovolume are given per culture jars.*

Culture jars	Temperature (°C)	Type of food	Number of juveniles of *Bulimina*	Number of specimens without chamber addition	Number of brocken specimens	Average length of the specimens at the start (µm)	Average length of the specimens (without non calcifying) at the end (µm)	Standard deviation (µm)	Average width of the specimens at the end (µm)	Standard deviation (µm)	Average biovolume ($*10^5$ µm^3)	Standard deviation ($*10^6$ µm^3)
I-C_1	10	freeze-dried *Chlorella*	75	18	15	93 ± 12	145	26	120	16	8.72	3.83
I-C_2	10	freeze-dried *Chlorella*	71	15	21		144	26	119	16	8.56	3.95
I-C_3	10	freeze-dried *Chlorella*	67	44	11		109	15	97	9	4.2	1.47
II-C	10	freeze-dried *Chlorella*	61	8	17		138	26	116	16	7.78	3.47
II-C+P	10	freeze-dried *Chlorella* + fresh *Phaeodactylum*	63	8	4		189	29	147	18	16.98	6.87
II-P	10	fresh *Phaeodactylum*	63	12	4		232	37	174	23	29.09	11.82
III-C	10	freeze-dried *Chlorella*	66	4	21		204	39	156	24	21.2	11.1
III-C+P	10	freeze-dried *Chlorella* + fresh *Phaeodactylum*	68	6	18		217	45	164	28	25.1	12.34
III-P	10	fresh *Phaeodactylum*	78	10	6		211	37	160	23	22.77	9.9
IV-P	10	fresh *Phaeodactylum*	79	20	2		148	34	122	21	9.61	5.54
IV-A	10	fresh *Amphiprora*	71	10	13		199	50	153	31	20.95	12.96
V-D_1	10	fresh *Dunaliella*	81	9	1	158 ± 32	245	38	182	24	33.61	13.57
V-D_2	10	frozen *Dunaliella*	80	8	14		231	38	173	24	28.9	13.85
V-P_1	10	fresh *Phaeodactylum*	82	7	16		284	54	206	33	51.24	26.02
V-P_2	10	frozen *Phaeodactylum*	80	3	12		262	36	192	22	39.66	14.11

CHAPITRE 4

EXPERIMENTAL PROTOCOLS TO MAINTAIN STABLE PHYSICO-CHEMICAL CONDITIONS FOR THE CULTURE OF DEEP-SEA BENTHIC FORAMINIFERA

Barras C. [1,2]*, Geslin E. [1], Michel E. [2], Gaultier M. [1], Duplessy J.-C. [2], Jorissen F. [1]

[1] Laboratory of Recent and Fossil Bio-Indicators (BIAF), UPRES EA 2644, Angers University, France, and Laboratory of Marine Bio-Indicators (LEBIM), Ile d'Yeu, France

[2] Laboratoire des Sciences du Climat et de l'Environnement (LSCE), CEA–CNRS-UVSQ, Gif-sur-Yvette, France

CHAPITRE 4

EXPERIMENTAL PROTOCOLS TO MAINTAIN STABLE PHYSICO-CHEMICAL CONDITIONS FOR THE CULTURE OF DEEP-SEA BENTHIC FORAMINIFERA

1. INTRODUCTION

The calcium carbonate calcified by foraminifera is a source of information for paleoceanographical studies (Sen Gupta, 1999a). The environmental conditions (temperature, salinity, productivity…) in which they calcified are recorded in the geochemical composition of their shell (isotopic composition, trace metals concentrations). Therefore they can be used as proxies to reconstruct paleoenvironments. The calibration of these proxies has been based on inorganic precipitation of calcite and/or on field samples such as foraminifera sampled in core tops, sediment traps, multinets (e.g. McCrea, 1950; O'Neil *et al*., 1969; Shackleton, 1974; Bouvier-Soumagnac and Duplessy, 1985; Kim and O'Neil, 1997; Lynch-Stieglitz *et al*., 1999; Mackensen and Bickert, 1999; Rathburn and De Deckker, 1997; Rosenthal *et al*., 1997; Lea, 1999). The culture of foraminifera in the laboratory appeared as an extra opportunity to improve the calibration of proxies. The interest of laboratory studies is the possibility to control all the parameters of the medium in which the foraminifera calcify, and moreover to control the variation of a single parameter while all the others are maintained stable. Theoretically, the effect of each environmental parameter on the foraminiferal calcite can be disentangled. Numerous researchers performed laboratory cultures on planktonic foraminifera in controlled conditions in order to understand the influence of these parameters (e.g. temperature, carbonate ion concentration…) on the geochemistry of foraminiferal tests (e.g. Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Spero and Williams, 1988; Nurnberg *et al*., 1996; Spero *et al*., 1996, 1997; Bemis *et al*., 1998; Russell *et al*., 2004). Laboratory experiments with shallow benthic foraminifera (Chandler *et al*., 1996; Toyofuku *et al*., 2000) and deep-sea benthic foraminifera (Wilson-Finelli *et al*., 1998; Havach *et al*., 2001; McCorkle *et al*., 2004; Hintz *et al*., 2004, 2006a, 2006b) for the calibration of proxies are scarcer. Most of the culture experiments were performed with planktonic foraminifera since they have the particularity of calcifying very rapidly large thick chambers, until gametogenesis appears. For this reason, the experiments are very short (e.g. between 6 and 15

days for Erez and Luz, 1983; Spero *et al*., 1997; Bijma *et al*., 1998) and consequently the maintenance of stable seawater conditions is facilitated. Unfortunately, the tests of benthic foraminifera are thinner therefore more chambers are required to obtain the same weight. However, the advantage to work on benthic foraminifera is that, unlike planktonic foraminifera, they are able to reproduce in the laboratory (e.g. Bradshaw, 1957, 1961; Angell, 1990; Stouff *et al*., 1999a; Hintz *et al*., 2004; Barras *et al*., submitted). For this reason, it is possible to perform measurements on a pool of foraminifera that calcified their entire test in controlled conditions and to obtain averaged values of isotopic and/or trace metal composition of foraminiferal shells. Shallow benthic foraminifera appear to be well adapted to laboratory conditions (e.g. Bradshaw, 1955, 1957, 1961; Chandler *et al*., 1996; Stouff *et al*., 1999a, 1999b, 1999c; Toyofuku *et al*., 2000; Ernst *et al*., 2005, 2006; Le Cadre *et al*., 2006), perhaps because they are used to a high environmental variability (e.g. high temperature and salinity ranges). Cultures of deep-sea benthic foraminifera are not yet very widespread, probably because they cannot be sampled easily and because little is know about their biology. For example, the type of food they require may be very specific, explaining the difficulty to obtain growth and reproduction in laboratory conditions. It is however very important to realise this proxy calibration in laboratory experiments for deep-sea benthic foraminifera, since they are frequently used for paleoceanographic studies and their geochemical composition could be influenced differently than most of planktonic or shallow benthic foraminifera.

The aim of this study was to define a protocol to culture deep-sea benthic foraminifera in the laboratory in controlled, stable geochemical seawater conditions, for a long period of time and in a simple (non expensive) way. We focused in this paper on the development of protocols for a study of the influence of seawater temperatures on the oxygen isotopic composition of deep-sea benthic foraminifera. We required specimens that calcified the totality of their test under stable geochemical conditions at different temperatures. In fact, measurements of the $\delta^{18}O$ composition of the shells are performed on a minimum weight of calcite. Next, we had to find a protocol to keep conditions stable over long-term experiments because incubated adult specimens needed first to reproduce and the produced juveniles had to grow enough to be able to measure their isotopic composition. We present here two types of protocols: a closed system (CS) and a Petri dish system (PD).

2. PARAMETERS INFLUENCING $\delta^{18}O$ COMPOSITION OF FORAMINIFERA

The oxygen isotopic composition of the foraminiferal shell is depending on the temperature of the seawater in which they calcify but also on the $\delta^{18}O$ value of the seawater (Shackleton and Opdyke, 1973; reviews in Rohling and Cooke, 1999; Wefer *et al*., 1999, Ravelo and Hillaire-Marcel, 2007). In the field, the $\delta^{18}O$ value of the seawater depends itself on the global climatic variations, such as ice volume, and on local variations, such as precipitation and evaporation. In laboratory conditions, we need to avoid any evaporation in order to keep the $\delta^{18}O$ of the experimental seawater constant. Therefore, the main parameters to control and keep stable in our experiments are temperature, salinity and $\delta^{18}O_{seawater}$. During the last decade, several studies (Spero *et al*., 1997; Zeebe, 1999) have demonstrated that the carbonate ion effect can also influence to a certain extent the stable isotopic composition of cultured planktonic foraminifera. Since we want to deconvolve the specific effect of temperature on the isotopic composition of foraminiferal shell, we also need to keep the carbonate chemistry of the experimental seawater as stable as possible. This is difficult because it depends on several reactions such as respiration of living organisms, precipitation and dissolution of $CaCO_3$. We decided to maintain invariable and check the following parameters: pH, alkalinity and Dissolved Inorganic Carbon or $\sum CO_2$ (see § 3.3. for precision about the measurements).

3. MATERIAL AND METHODS

3.1. Culture of deep-sea benthic foraminifera

In this study, we focus mainly on the culture of the species *Bulimina marginata* (*marginata* and *aculeata* morphotypes) because previous experimental cultures of deep-sea benthic foraminifera reported a good adaptation of the *Bulimina* species to laboratory conditions (Wilson-Finelli *et al*., 1998; Havach *et al*., 2001; Bernhard *et al*., 2004; Hintz *et al*., 2006a, 2006b; McCorkle *et al*., 2004; Barras *et al*., submitted). The study realised by Barras *et al*. (submitted) aimed to determine the most favourable conditions to obtain reproduction and growth of *Bulimina marginata* in a short period of time. The range of temperatures and food types tested in our controlled experiments was fixed according to these conclusions. Moreover, relatively high densities of living *Bulimina* were found in the sediments sampled for our experiments, facilitating the task to select the numerous living specimens needed for the experiments. However, we also used assemblages of other species of benthic foraminifera

in our experiments such as *Bolivina subaenariensis* and *Hyalinea balthica* to try to obtain reproduction and growth of other species than *Bulimina marginata*.

3.1.1. Foraminiferal sampling and preparation

Living specimens of benthic foraminifera were sampled at two deep-sea stations in the Bay of Biscay, north-east Atlantic. Both stations are located in the axis of Cap Breton canyon. Stations G (43°40'N-1°37'W) and K (43°37N-1°43W) are respectively situated at 450 and 650 m water depth. The bottom water temperatures at stations G and K are ~11.2 and ~10.5°C, respectively, and the salinity is around 36‰.

In June 2006, numerous cores were collected using a multi-tube corer (Barnett *et al*., 1984). The top 2 centimetres of sediment, containing the largest number of living foraminifera (more than 80% of the fauna at these stations; Hess *et al*., 2005), were sampled with a spoon and placed in polyvinyl chloride transparent bottles previously filled with siphoned bottom water from the same core. All the sediment samples were transported back to Angers laboratory in cool boxes to maintain cool conditions. In the laboratory, the cores were all kept at 10°C, at a salinity of 36‰, without addition of food. The sediment constituted a stock of living foraminifera that could be used for the experiments after a relatively long period of time (8 to 13 months). The foraminiferal assemblages at these locations are dominated by *Bolivina subaenariensis, Bulimina marginata* and *Hyalinea balthica* (Hess *et al*., 2005; Hess *et al*., in prep.).

In order to obtain foraminiferal calcite formed under stable conditions, we followed two different strategies (Figure 4.1): strategy 1) incubate labelled adult specimens, wait for reproduction in the controlled conditions; next, their juveniles have to grow enough to obtain a quantity of calcite sufficient for geochemical analyses, and strategy 2) incubate small labelled juvenile specimens and wait that they calcify new chambers in equilibrium with the controlled seawater. The first strategy allows to measure the $\delta^{18}O$ composition of entire shells calcified under controlled conditions. For the second strategy, it is necessary to evaluate the proportion of the isotopic signal that corresponds to the initial chambers calcified in uncontrolled conditions. Otherwise, it is possible to measure individual chambers by laser ablation techniques. The advantage of the second strategy is that no reproduction is required, so that the experimental periods can be shorter.

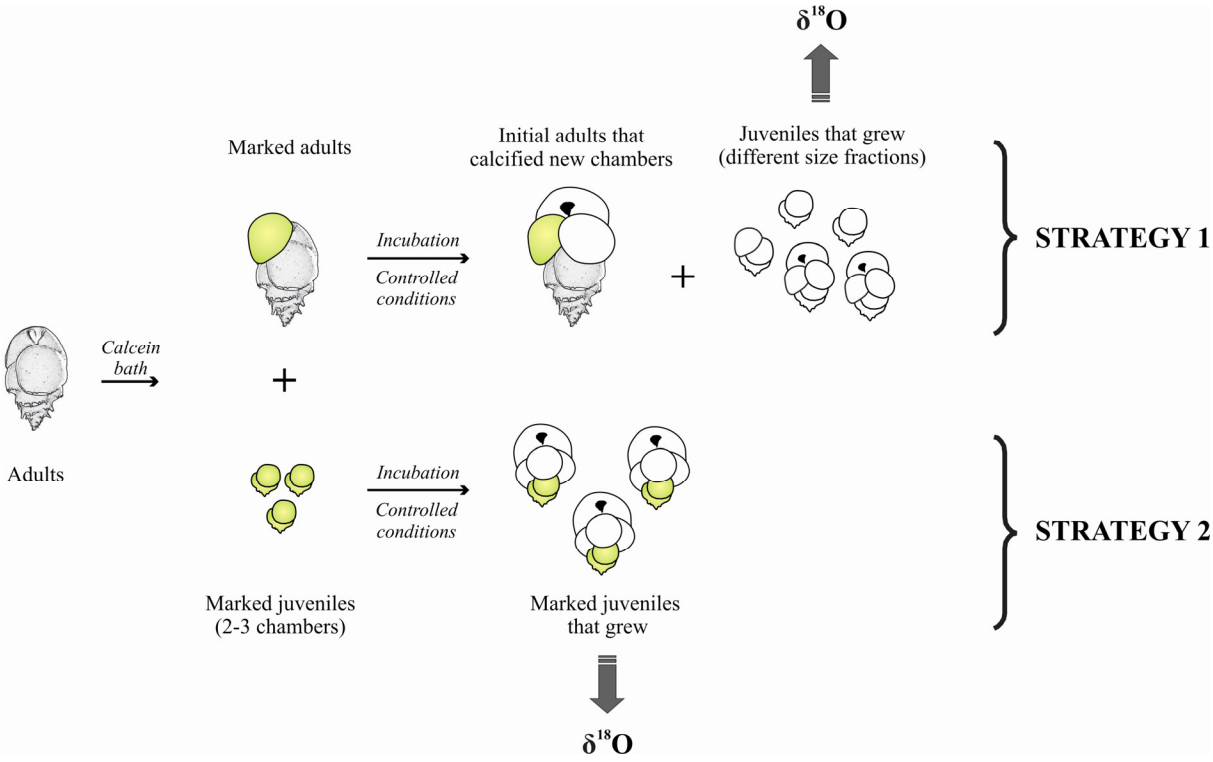


Figure 4.1: Description of strategies 1 and 2 tested to produce calcite in controlled conditions.

Strategy 1:

Before foraminifera could be incubated in the experiments, they had first to be labelled. In fact, we need to be able to distinguish between calcium carbonate precipitated in our controlled conditions and the pre-existing foraminiferal calcite. We used the fluorescent compound calcein (Bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) (see Chapter 3). Bernhard *et al*. (2004) were the first authors to use this probe with foraminifera. The advantage of this $CaCO_3$ labelling is that it is permanent, therefore labelled juvenile specimens incubated at the beginning of the experiment (and their initial size) can be identified by their fluorescence under the epifluorescent stereomicroscope. In our controlled experiments, newly formed specimens will not be fluorescent. Sediment samples were sieved at 150 µm before the beginning of the experiments. The larger fraction (> 150 µm), containing adult foraminifera, was incubated in a 10 $mg.l^{-1}$ solution of calcein (Fluka, Sigma Aldrich) in microfiltrated seawater (salinity ~ 36‰) in a Duran bottle at 10°C (preservation temperature close to *in situ* temperature). After approximately one month of calcein incubation with some food added (freeze-dried *Chlorella* sp., green algae), the sediment was cleaned using 150 and 38-µm mesh sieves with seawater at 10°C and both fractions were

observed under the epifluorescent stereomicroscope (Olympus SZX-12 stereomicroscope equipped with epifluorescence optics, excitation at 470 nm, emission at 500 nm).

In the larger fraction, adult specimens of all species that calcified at least one new chamber (which appeared fluorescent under the epifluorescent stereomicroscope) were picked (upon a tray of ice, to maintain cool conditions). Figure 4.2 presents specimens of *B. marginata*, *B. subaenariensis*, *H. balthica* and *U. peregrina* with marked chambers. In order to distinguish accurately between living and dead specimens, the selected specimens were kept in a glass Petri dish in the presence of *Chlorella* sp. for one or two days. It was then possible to discriminate the actively living foraminifera, which presented a green cytoplasm (because of the ingestion of *Chlorella*), from the inactive specimens which stayed totally transparent (Barras *et al*., submitted). The green specimens could then be incubated in the different experimental systems.

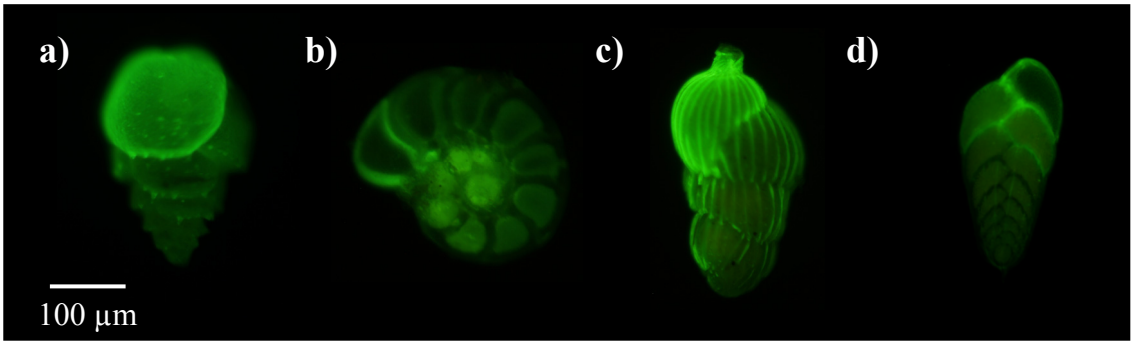


Figure 4.2: Pictures (epifluorescent stereomicroscope) of specimens that calcified new chambers in the presence of calcein. a), b) and c) specimens of B. marginata*,* H. balthica *and* U. peregrina*, respectively, with the last chamber marked; and d) specimen of* B. subaenariensis *with the last 3 chambers marked. The fluorescence observed in the rest of the specimen of* H. balthica *is possibly due to seawater vacuoles inside the cytoplasm that contain calcein.*

Strategy 2:

The small labelled juveniles used for this strategy were born in the presence of sediment in the calcein bath. They were picked from the 38–100 µm fraction. We used labelled juveniles (1) to distinguish unlabelled foraminifera that were born in our controlled experiments from the juveniles incubated at the beginning, and (2) to distinguish between the calcite of these juveniles formed under the controlled conditions and the calcium carbonate formed before.

At the end of the experiments, we would obtain growth of marked juveniles. To be able to estimate the proportion of calcite formed in our controlled conditions compared to the total quantity of calcite, we decided to calculate the volume of calcite of the specimens (volume of calcite = external volume of the shell – internal volume of the shell), taking into account the thickness of the shell wall, at the beginning and at the end of the experiments. The wall thickness of *B. marginata* was estimated at 8 µm, estimation based on SEM pictures of several juveniles and adult specimens (Plate 4.1). The shape of *B. marginata* can be considered as an ellipsoid. Therefore, we calculated the volume of the specimens using the following formula:

$$Test\ volume\ (\mu m^3) = 4/3 * \pi * (Length / 2) * (Width / 2)^2$$

The size and volume of calcite of the marked juveniles added at the beginning of the experiment were determined by the measurements of 85 specimens of the pool of juveniles used. Their average size was 94±10 µm in length and 79±11 µm in width and their average calcite volume was $(1.47\pm0.39)*10^5$ μm^3.

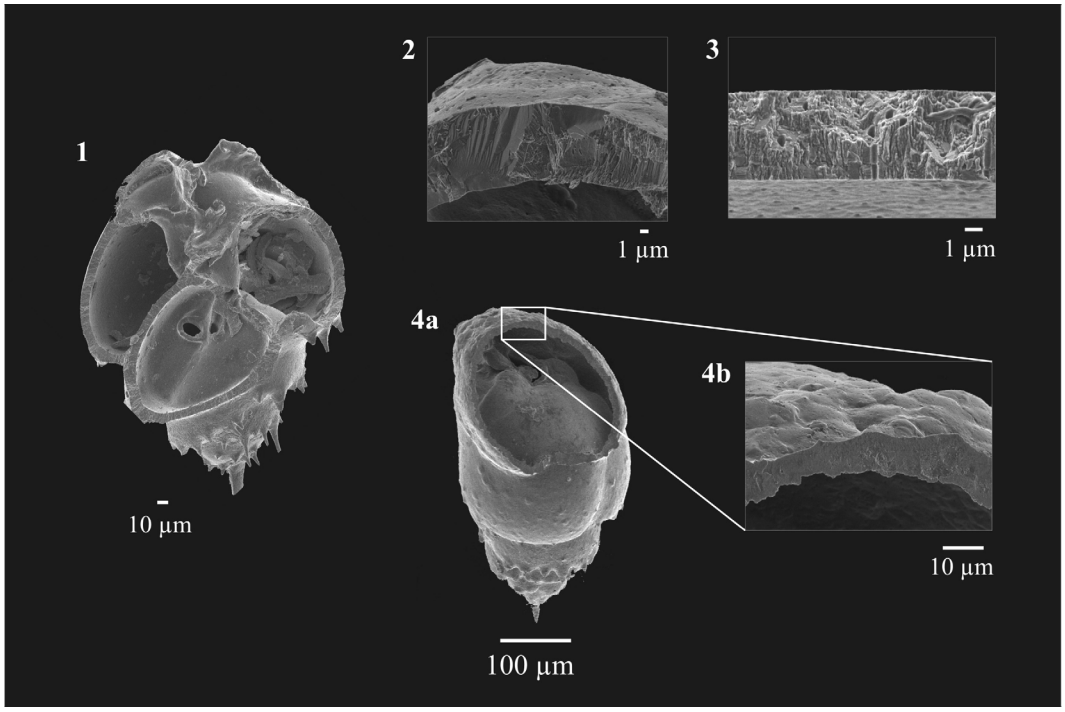


Plate 4.1: Wall cross sections of B. marginata*. Fig. 1: Longitudinal section of an adult sampled in the Bay of Biscay; Fig. 2: Wall cross section of a juvenile born in culture; Fig. 3: Wall cross section of a chamber calcified by an adult in culture; Fig. 4 a-b: Specimen that calcified the last chamber against the wall of the Petri dish.*

3.1.2. Foraminiferal feeding

The food particles presented to the foraminifera in the experiments were fresh diatoms, *Phaeodactylum tricornutum*. These fresh diatoms were chosen because their efficiency as food for *Bulimina marginata* was demonstrated by Barras *et al*. (submitted). Murray (1963), Wilson-Finelli *et al*. (1998), Havach *et al*. (2001) and Le Cadre and Debenay (2006) also used fresh diatoms for different culture experiments with foraminifera. *Phaeodactylum tricornutum* was cultivated in a F/2 medium with addition of sodium metasilicate in Erlenmeyer flasks, at 20°C and under 12:12h light-dark cycle. Diatoms were sampled fresh before lag phase.

3.1.3. Culture medium

The foraminiferal culture medium was microfiltrated (0.45 µm) natural seawater sampled in the Bay of Biscay. The seawater stock was stored in tight **200-litres tanks** and sampled when seawater was required for the experiments (no experiment was directly connected to them). The problem was that the removal of water from the tank went together with the introduction of ambient air inside the tank. Now, this air, containing water vapour with a different $\delta^{18}O$ composition than the seawater, needed to be dried before entering the tank in order to keep constant seawater $^{18}O/^{16}O$ ratio for all the experiments. For this reason, the 200-litres tank was connected to a water vapour trap (Figure 4.3). During seawater sampling, the air introduced flowed through the trap that condensed the water vapour so that only dried air was in contact with the seawater.

In order to prevent any microhabitat effect, all the experiments described in this paper were performed without artificial or natural sediment. Laboratory experiments demonstrated that *Bulimina marginata* can reproduce without sediment (Barras *et al*., submitted), and also that other species such as *Bolivina subaenariensis* and *Hyalinea balthica* can live and calcify new chambers without sediment (Barras, personal observation).

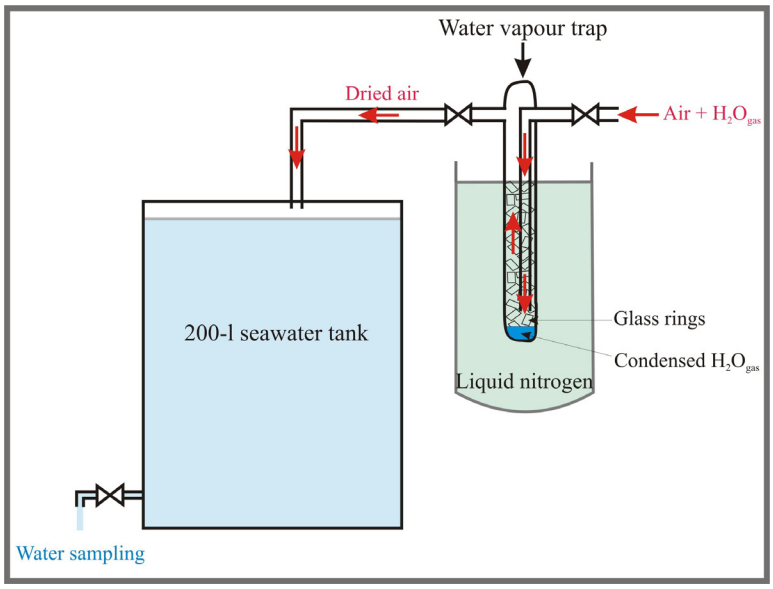


Figure 4.3: Diagram of the water vapour trap used to keep the isotopic composition of the 200-litres seawater tank constant through time.

3.2. System setup

We present two different types of setup to maintain stable conditions during the culture of deep-sea benthic foraminifera: 1) a closed system (CS) and 2) a Petri dishes system (PD). The interest to set up two different systems was to maximise the chance to be successful in the culture part and in the maintenance of stable physico-chemical conditions. The main difference between the two systems is that observations were possible during the experiments with the Petri dish system. Experiments were performed in both systems to determine the influence of temperature on the oxygen isotopic composition of the foraminifera.

3.2.1. The closed system (CS)

The closed system described in this paper is inspired by the system developed by Hintz *et al.* (2004). It is based on the recirculation of microfiltrated natural seawater. With the help of a pump, the seawater flows from a **20-litres tank,** after through different **experimental bottles** (in which living foraminifera were added), come back to the 20-litres tank, and the loop start again (Figure 4.4). This system is named "closed system" since the water circulates through a closed loop. The system is water tight (no connection with the atmosphere) to avoid evaporation and to keep the salinity constant. However, it is not totally gas tight (type of tubing). Each closed system contains a large volume of water so that putative variations of the different physico-chemical parameters are diluted and that water samples, to control the

parameters, can easily be collected during the experiments without disturbance. The circulation of the water aims also to avoid stratification of the water column in the experimental bottles.

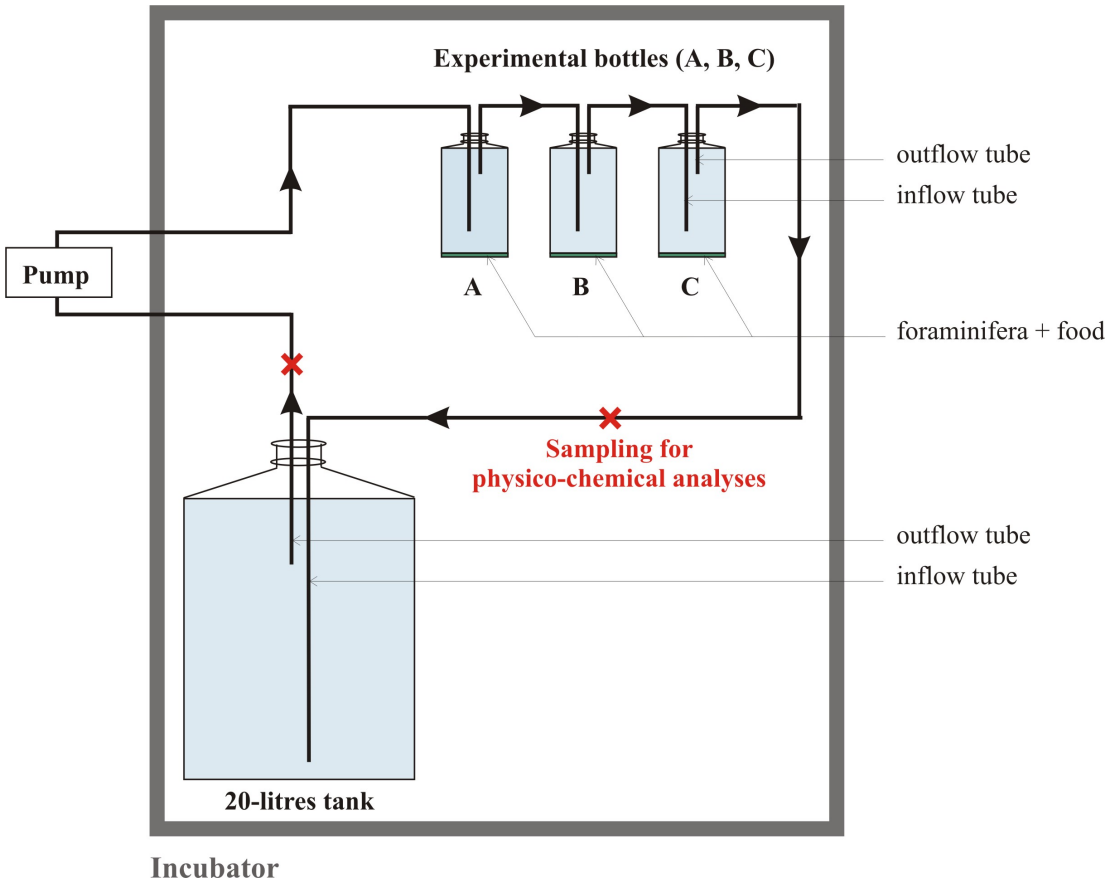


*Figure 4.4: Diagram of a closed system (CS). The circulation of the seawater through the **20-litres tank** and the **experimental bottles** is indicated by arrows. Foraminifera are cultured at the bottom of experimental bottles A, B and C (3 bottles in the case of CSI, 2 bottles for CSII). The bold grey rectangle represents the incubator; the pump is located outside and is connected (in parallel) to other closed systems. The two red crosses represent the water sampling locations for physico-chemical analyses for CSI; for CSII, water was only sampled at the outlet of the experimental bottles.*

In this paper, we present two groups of closed system experiments, which are called CSI (first group of closed systems) and CSII (second group of closed systems). CSI corresponds to 4 closed systems maintained at 7.9 (CSI-7.9), 10.1 (CSI-10.1), 12.7 (CSI-12.7) and 14.7°C (CSI-14.7), and CSII corresponds to 6 closed systems maintained at 4.1 (CSII-4.1), 6.0 (CSII-6.0), 9.3 (CSII-9.3), 11.6 (CSII-11.6), 17.2 (CSII-17.2) and 19.3°C (CSII-19.3). Within

one closed system, a certain number of experimental bottles containing the living foraminifera can be connected in series as is illustrated on Figure 4.4.

One closed system consisted of one 20-litres opaque tank (Length×Depth×Height = 295×245×392 mm) and 2 or 3 one-litre experimental bottles (L×D×H = 95×76×185 mm, with a membrane seal and a stopper) containing the living foraminifera. Both containers (20-litres tanks and experimental bottles) were made of high density polyethylene (HDPE). The water circulated using a peristaltic pump (ISMATEC ICP) fixed at 1 $ml.min^{-1}$ for CSI and 3 $ml.min^{-1}$ for CSII. These flow rates correspond to a total renewal of the water of one experimental bottle in 16 and 5 hours for CSI and CSII, respectively. For CSI, silicon tubing (3.2 mm inner diameter and 4.8 mm outer diameter) was used to connect the different containers and the pump. For CSII, Tygon® tubing (FEP SE-200, 3.2 mm inner diameter and 6.4 mm outer diameter) was used because we wanted to obtain gas tight conditions. The stopper of the 20-litres tanks and the membrane seal of the experimental bottles were perforated and female-male luer locks allowed to connect tubes outside and inside containers. The inflow tubes were longer than the outflow tubes inside all containers (Figure 4.4) (1) to create a seawater circulation inside the containers and avoid stratification of the water column and (2) in order to avoid resuspension of foraminifera or food that could then flow through the tube into the next experimental bottle. In the 20-litres tanks, the inflow and outflow tubes were around 3 cm and 13 cm above the bottom, respectively. For the experimental bottles, the inflow and outflow tubes were 5 cm and 12 cm above the bottom tubes, respectively.

The 20-litres tanks as well as the experimental bottles were filled up with medium (natural microfiltrated seawater) before the start of the experiments. Air was purged from the tubes at the beginning of the experiments. Two stocks of natural microfiltrated seawater, from the Bay of Biscay, stored in 200-litres tanks (sheltered from light and water tight) were used for all the experiments. The water used for CSI experiments was surface seawater (stored in Tank200-I) whereas the water used for CSII experiments came from 100 m water depth (stored in Tank200-II).

The systems (20-litres tank + experimental bottles) were then installed inside the incubator at the desired temperature some days before the introduction of the foraminifera and the start of the experiments (Figure 4.4). Only the pump was left outside of the incubators and served for all the closed systems, with one canal used per system (parallel connection). No connection linked the various closed systems to each other.

Only the strategy 1 (introduction of adult specimens) was employed in the closed systems. The well green-stained and labelled adult foraminifera (> 150 µm) coming from the calcein baths were separated in equal quantities of specimens per species so that the chance to obtain reproduction was equal in all the experimental temperatures of one group of systems (CSI or CSII). Foraminifera were led progressively to the desired experimental temperature before incubation in order to avoid thermal stress. When the required seawater temperature was reached, the adult foraminifera were added directly at the bottom of the experimental bottles with a 27-cm long glass Pasteur pipette.

In CSI, 3 experimental bottles (A to C) were connected to each system (Figure 4.5). Experimental bottles A and B of the 4 systems (CSI-7.9, CSI-10.1, CSI-12.7 and CSI-14.7) contained each 100 adult specimens of *Bulimina marginata* (~90% *marginata* and 10% *aculeata* morphotypes). They were separated from the rest of the fauna to avoid any competition with the other species. The experimental bottles C contained a mixed assemblage of labelled deep-sea benthic foraminifera: *Bolivina subaenarensis* (300 specimens), *Hyalinea balthica* (30 specimens), *Uvigerina peregrina* (8 specimens) and really few specimens of *Ammonia beccarii*, *Bolivina quadrilatera*, *Cassidulina carinata* and *Amphicorina scalaris*.

Two bottles (A and B) were connected to each system of CSII (Figure 4.5). Only *B. marginata* (80% *marginata* and 20% *aculeata* morphotypes) were incubated in experimental bottles A. In total, 100 labelled living specimens were added in the experimental bottles A of the colder systems (e.g. CSII-4.1, CSII-6.0 and CSII-9.3) and 150 specimens were added in the bottles A of the warmer systems (e.g. CSII-11.6, CSII-17.2 and CSII-19.3). In the experimental bottles B, a small number of specimens (1 to 15 individuals) were incubated from each of several species such as *B. subaenariensis*, *U. peregrina*, *H. balthica*, *Gyroidina umbonata*.

In order to feed the foraminifera from CSI and CSII experiments, fresh diatoms were first concentrated by centrifugation. The overlying medium solution was removed and the concentrate was mixed with seawater from the system. The C/N ratio of the added food was 7.4. This shows that the marine organic matter used as diet was fresh. For CSI and CSII, food was added twice in 3 months with the help of a 27-cm long Pasteur pipette: once at the beginning of the experiment for both groups and a second time after 3 weeks for CSII and after one month for CSI. Two to three hours after the incubation of food and foraminifera, the pump was switched on so that the diatoms had enough time to settle down at the bottom of the

bottles. In the following hours, the systems were checked and all eventual leaks were repaired.

All the closed systems were run for approximately 3 months (Figure 4.5).

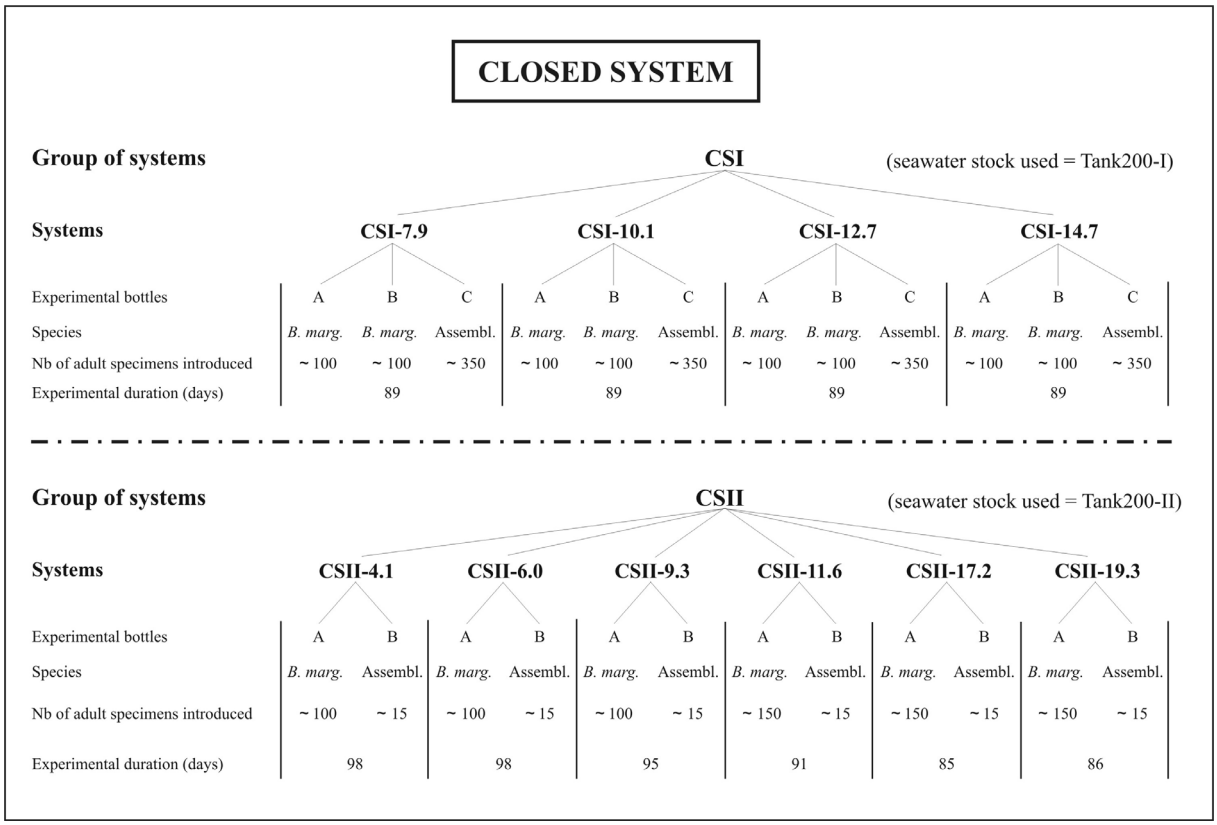


Figure 4.5: Summary of the experimental conditions (species and number of specimens introduced, and duration of the experiments) in all the closed systems (CSI and CSII). For these experiments, strategy 1 was applied.

3.2.2. Petri dish system (PD)

We decided to develop a second type of system to make observations more easily possible during the experiments, which was virtually impossible with the closed system. In the PD setup, experiments take place in 120-ml polypropylene Petri dishes (diameter of 75 mm and height of 30 mm) filled up until 5 mm under the top. They were equipped with lids exactly adapted to the basal part of the Petri dishes. A piece of Parafilm® "M", tight to liquids, was placed in between to reduce the risk of evaporation. To maintain stable physico-chemical conditions, half of the seawater (~ 60 ml) was replaced in each Petri dish every 3 to 4 days (twice per week) to avoid the impact of evaporation or pH decrease on experimental stability (Figure 4.6). This manipulation was realised under the stereomicroscope (LEICA $MZ12_5$) with a 5-ml sterile syringe equipped with a needle tip in order to check that no foraminifera

was removed from the Petri dish. A tray of ice was used to maintain cool conditions during manipulation. The "new" seawater (coming from the 200-l tank) was stored in a Duran bottle and placed in the incubator at the desired temperature. Once this temperature was reached, the "new" seawater was added to the Petri dishes.

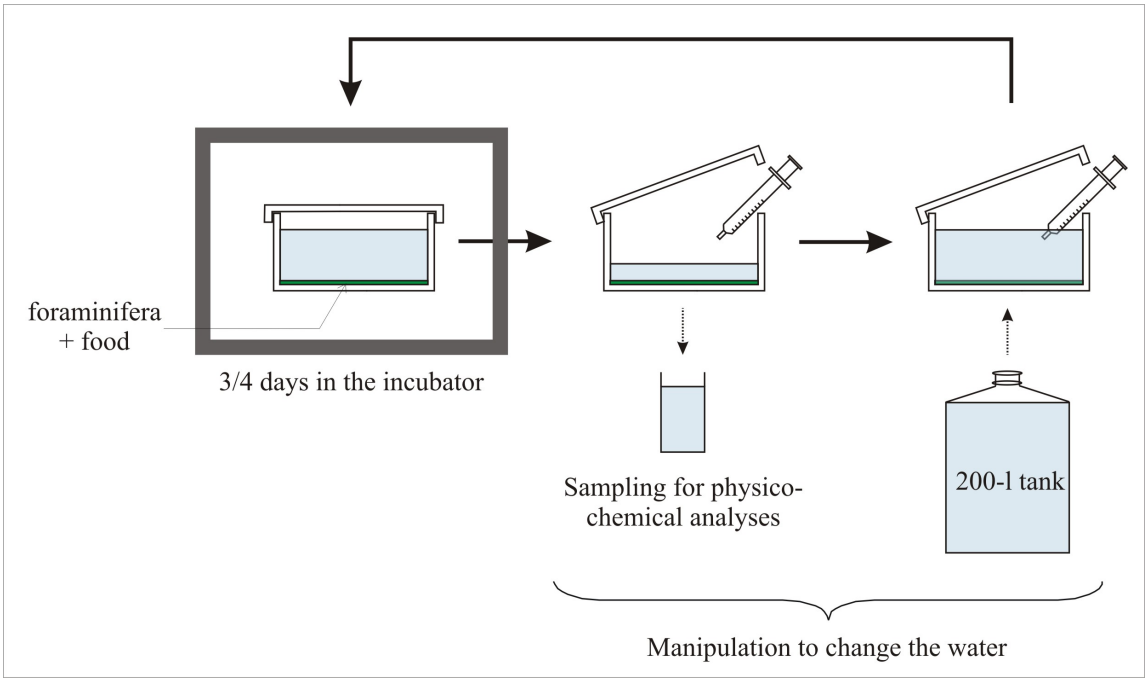


Figure 4.6: Diagram of the Petri dish system (PD). The grey rectangle represents inside the incubator. Every 3 to 4 days, half of the seawater inside the Petri dish is replaced by seawater from a 200-litres tank. The seawater removed is used for physico-chemical analyses.

For our experiments aiming to test the impact of temperature on the $\delta^{18}O$ of foraminifera, one Petri dish was each one placed of the six incubators fixed at 7.9 (PD-7.9), 10.2 (PD-10.2), 12.7 (PD-12.7), 14.7 (PD-14.7), 15.7 (PD-15.7) and 13.0°C (PD-13.0). Experiments PD-7.9, PD-10.2, PD-12.7 and PD-14.7 were placed in the same incubators as the closed systems CSI and both experiments were run at the same time.
Natural microfiltrated seawater used to run the PD experiments was coming from Tank200-I. Seawater was homogenised before sampling for addition to the Petri dishes.

For PD-7.9, PD-10.2, PD-12.7 and PD-14.7 experiments, strategy 1 (introduction of adult specimens) and strategy 2 (introduction of labelled juveniles) were performed within each Petri dish (Figure 4.7). This means that, at the beginning of the experiments, living adult specimens expected to reproduce and labelled juveniles expected to grow were introduced

together in each Petri dish. For PD-15.7 and PD-13.0, only strategy 1 was performed. The specimens incubated in these experiments were added directly in the Petri dishes.
Experiments PD-7.9, PD-10.2, PD-12.7 and PD-14.7 were containing exclusively specimens of *Bulimina marginata*. In total 30 green-coloured and labelled adult specimens of *B. marginata* (*marginata* morphotype, strategy 1) as well as 250 small labelled juvenile specimens of *B. marginata* (both morphotypes, strategy 2) were added in each Petri dish (Figure 4.7). For PD-15.7, 190 green-coloured labelled adult specimens of *B. marginata* (50% *marginata* and 50% *aculeata* morphotypes) were incubated and 45 specimens of *B. marginata* (*marginata* morphotype) in PD-13.0 (Figure 4.7).

For PD, diatoms were not concentrated before addition. The quantity of water is much smaller than in the closed systems so we feared a rapid decrease of pH if too much organic matter was added suddenly. Only 3 ml of fresh *P. tricornutum* was sampled, centrifuged, the supernatant removed and mixed with seawater from the system before addition. Therefore the quantity of food added was lower than for the closed systems but the feeding frequency was higher (once every two weeks).
Experiments PD-7.9, PD-10.2, PD-12.7 and PD-14.7 lasted for 3.5 months. Experiments PD-15.7 and PD-13.0 last respectively for 59 and 43 days.

PETRI DISH SYSTEM

Petri dishes	**PD-7.9**	**PD-10.2**	**PD-12.7**	**PD-14.7**	**PD-15.7**	**PD-13.0**
Strategy	1 & 2	1 & 2	1 & 2	1 & 2	1	1
Species	*B. marg.*	*B. marg.*	*B. marg.*	*B. marg.*	*B. marg.*	*B. marg.*
Nb of adult specimens introduced	30	30	30	30	190	45
Nb of labelled juvenile specimens introduced	~ 250	~ 250	~ 250	~ 250	-	-
Experimental duration (days)	108	108	108	108	59	43

Figure 4.7: Summary of the experimental conditions (strategy employed, species and number of specimens introduced, and duration of the experiments) in the Petri dish system (PD).

3.3. Measurements of physico-chemical parameters

3.3.1. Devices, treatment of samples and precisions

The temperature stability was monitored by thermometers (Testo-174 or 175-T2) with a precision of 0.1°C. These devices recorded the temperature inside the incubators every 10 minutes during the experiment periods.

Precise salinity measurements at the beginning and at the end of CSI closed systems were performed at the laboratory of Roscoff (France) with a Guildline Portasal Salinometer with a precision of ± 0.002‰. Additionally, salinity measurements were performed every week for CSI and CSII, and every 3-4 days for PD, using a Conductimeter WTW 330i. A vial of 20 ml of seawater sampled in the experiment was closed and left outside the incubators for 2 to 3 hours to reach ambient temperature before the measurement. The conductimeter was calibrated with a solution at 1413 $\mu S.cm^{-1}$. No standard solutions were available in the range of our salinity values. Next, we used salinity measurements performed in Tank200-I and Tank200-II as references to standardise the salinity measurements of the seawater in the systems and remove the variations caused by the temporal variability of the conductimeter. The water tightness of the 200-l tank was proven by similar salinity at the start and at the end of the experimental period. The precision of the measurements performed with the conductimeter is ± 0.1‰.

For pH measurements and alkalinity titrations, a combined glass electrode Ag/AgCl connected to a pHmeter CG 820 (Schott) was used for CSI and PD systems, and a Methrom electrode connected to the Methrom (785 DMP Titrino) titration device was used for CSII.
For pH measurements, electrodes were calibrated using 7.01 and 10.01 (± 0.01) NIST buffer solutions. PH was measured in the same sample and at the same frequency as salinity. The precision of the devices can be estimated from the repeatability of the measurements for a given period of time in the 200-l tanks considered as standards (Tank200-I and Tank200-II). For both electrodes, the standard deviations are ± 0.03 and maximum ranges of variation (difference between the lowest and highest value recorded) were 0.15 and 0.12 for the pHmeter and the titration device, respectively.
Alkalinity was determined weekly by Gran titration (Gran, 1952) for CSI, CSII, Tank200-I and Tank200-II, and biweekly for the supernatant of the Petri dishes. The alkalinity was measured manually for CSI, PD and Tank200-I with 0.5 N hydrochloric acid. Three replicates

of 20 ml samples of water were analysed for each CSI system and in Tank200-I (water added to the Petri dishes). Due to the time-consuming character of manual titrations, alkalinity was not measured at the outlet of the experimental bottles for CSI but only at the outlet of the 20-litres tank. The alkalinity measurements were not replicated for the supernatant water (removed after 3-4 days) of the Petri dishes since the volume was insufficient. For CSII, 10 ml of seawater was titrated with a 0.1 N HCl certified using Methrom titration device. For these titrations, the electrode was calibrated with NIST buffer solutions at 7.01 and 4.01 (± 0.01). Measurements in Tank200-I (manual titrations) and in Tank200-II (Methrom titrations) give a standard deviation of the measurements of 23 and 14 $\mu mol.l^{-1}$ and a maximum range of 118 and 50 $\mu mol.l^{-1}$, respectively. The maximum error obtained for replicate measurements was 2% for manual titrations and 0.7% for Methrom titrations, which corresponds to the precision of the measurements.

We measured DIC on 2-ml water samples poisoned with saturated mercuric chloride ($HgCl_2$) after microfiltration at 0.45 µm. The samples were stored in glass amber tubes at ambient temperature. The measurements were done in the EPOC laboratory in Bordeaux (France) with a flow injection analysis system (FIA; Hall and Aller, 1992). The standard solutions used to calibrate the measurements where solutions prepared with $NaHCO_3$ at concentrations of 2, 2.2, 2.4, 2.6, 2.8 and 3 $mmol.kg^{-1}$. The maximum standard deviation of the replicate measurements was ± 0.13 $mmol.kg^{-1}$ which corresponds to an error of 5%.
Unfortunately the measurements were not precise enough for the monitoring of our systems (variability of the measurement was too high to record possible variations in our systems). These data are not presented in this paper. Instead, DIC was calculated using the CO_2sys program (Lewis and Wallace, 1998) with the dissociation constants reported by Roy *et al*. (1993) and Dickson (1990). Knowing pH and alkalinity, this program allows to calculate the rest of the carbonate parameters. Although we are aware that measuring pH and alkalinity is the most simple but not the most accurate method to fully determine the carbonate system, we used them to roughly estimate the Dissolved Inorganic Carbon (total CO_2) variations through the experiments.

Water samples for $\delta^{18}O$ analyses were microfiltrated at 0.45 µm and stored in glass amber tubes at 4°C to avoid any evolution of the water chemistry. Samples were analysed with a Gas Bench at the LSCE in Gif-Sur-Yvette (France). For this analysis, carbon dioxide is introduced

in the analysing tube containing the seawater and left at constant temperature so that the CO_2 isotopic composition of the gas equilibrates with the one of the seawater. After 18 hours, a special two-hole needle blows helium into the tube. The outflow is purified through a chromatograph and the fraction containing the carbon dioxide is injected in the mass spectrometer source where are measured the masses 44 ($^{12}C^{16}O^{16}O$) and 46 ($^{12}C^{18}O^{16}O$). The masses ratio 46/44 allows to measure the $^{18}O/^{16}O$ ratio of the seawater by comparison to the signal of a standard with a known isotopic composition.

Unfortunately, seawater $\delta^{18}O$ data were not available for the redaction of this manuscript. Therefore, we used an average $\delta^{18}O$ value of surface seawater from the Bay of Biscay of 0.55‰. In the meantime, measurements of surface seawater sampled in the Bay of Biscay, performed by A. Mackensen (AWI, Germany), gave a mean value of 0.52 ± 0.08‰ (Table 4.1). The value of the seawater at the end of the CSII experiments (after 3 months in the system) was in average 0.63 ± 0.05‰, taking the seawater of all experiments together (Table 4.1). Therefore the $\delta^{18}O_w$ in the systems stay relatively stable during the experiments and values are comparable whatever the temperature of the system.
In the rest of the manuscript, we decided to keep the value of 0.55‰ for the calculations. The values will be corrected when measurements of the seawater of each experiment will be available.

Seawater samples	$\delta^{18}O_w$ (‰ SMOW)
Bay of Biscay surface seawater	0.55 0.43 0.58
CSII-4.1	0.65 0.53
CSII-6.0	0.62 0.62
CSII-9.3	0.62 0.68
CSII-11.6	0.57 0.68
CSII-17.2	0.66 0.66
CSII-19.3	0.62 0.61

Table 4.1: $\delta^{18}O$ values of surface seawater of the Bay of Biscay and of the seawater used for the CSII experiments (sampled at the end of the experiments).

3.3.2. Water sampling location and frequency

For CSI, water was sampled at two different locations in the closed system: (1) at the outlet of the 20-litres tank, to check the physico-chemical conditions of the water before the experimental bottles, and (2) at the outlet of the third experimental bottle C to check the composition of the water after its flow through the three experimental bottles (Figure 4.4). The aim was to check if the salinity and pH of the seawater were constant within the experimental setup. Since no difference was measured for the physico-chemical parameters between the two sampling locations in CSI, water was only sampled at the outlet of experimental bottles B for CSII, representative for the conditions of the water within the bottles. For CSI, the rest of the parameters were measured in the water at the outlet of the 20-litres tank. In practice, water was sampled by plugging a syringe to the tube disconnected briefly from the system. Measurements were realised every week for both CSI and CSII. During sampling, the pump was temporarily stopped.

For the Petri dish system, the water that was removed every 3 to 4 days (supernatant) was used for salinity, pH, alkalinity and $\delta^{18}O$ measurements. These parameters were also measured twice per week in the water coming of the 200-l tank (Tank200-I) added to the Petri dishes.

4. RESULTS

4.1. Stability of the systems

Table 4.2 summarises all the physico-chemical parameters measured (temperature, salinity, pH and alkalinity) in order to check the stability of the systems. The survey of each parameter is detailed in the following paragraphs.

		Temperature (°C)				Salinity (‰)				pH				Alkalinity (µmol/l)			
		n	Average	Range	SD	n	Average	Range	SD	n	Average	Range	SD	n	Average	Range	SD
Group of systems CSI	CSI-7.9	90	7.9	7.8 - 8.2	0.1	26	35.8	35.8 - 35.9	0.1	27	7.94	7.84 - 8.00	0.04	39	2288	2234 - 2324	24
	CSI-10.1	90	10.1	10.0 - 10.6	0.1	26	35.8	35.8 - 35.9	0.1	27	7.96	7.86 - 8.02	0.04	39	2291	2239 - 2324	21
	CSI-12.7	90	12.7	12.6 - 13.1	0.1	26	35.9	35.8 - 36.0	0.1	27	7.98	7.86 - 8.03	0.04	37	2307	2244 - 2367	34
	CSI-14.7	90	14.7	14.6 - 14.9	0.1	26	35.8	35.8 - 36.0	0.1	27	7.96	7.86 - 8.00	0.04	39	2307	2220 - 2384	31
Group of systems CSII	CSII-4.1	98	4.1	3.6 - 9.5	1.1	12	35.8	35.7 - 35.9	0.1	12	7.80	7.71 - 7.91	0.07	25	2492	2465 - 2525	13
	CSII-6.0	98	6.0	5.7 - 10.3	0.5	12	35.8	35.7 - 35.9	0.1	12	7.80	7.70 - 7.91	0.08	24	2488	2469 - 2511	12
	CSII-9.3	95	9.3	8.0 - 13.4	0.7	12	35.8	35.7 - 35.9	0.1	12	7.78	7.66 - 7.92	0.09	23	2488	2469 - 2511	13
	CSII-11.6	91	11.6	11.5 - 14.3	0.3	11	35.8	35.8 - 35.9	0.1	11	7.80	7.66 - 7.95	0.10	21	2489	2469 - 2511	11
	CSII-17.2	85	17.2	17.1 - 18.4	0.2	10	35.8	35.7 - 35.8	0.1	10	7.77	7.63 - 7.95	0.10	20	2485	2446 - 2508	19
	CSII-19.3	86	19.3	19.2 - 19.8	0.1	10	35.8	35.7 - 35.8	0.1	10	7.80	7.68 - 7.95	0.09	20	2483	2456 - 2508	16
Petri dish system	PD-7.9	108	7.9	7.8 - 8.1	0.1	30	35.9	35.8 - 35.9	0.1	30	7.92	7.80 - 8.11	0.06	29	2283	2224 - 2382	36
	PD-10.2	108	10.2	10.0 - 10.6	0.1	30	35.8	35.8 - 35.9	0.1	30	7.92	7.80 - 8.12	0.06	30	2285	2173 - 2408	43
	PD-12.7	108	12.7	12.6 - 13.2	0.2	30	35.9	35.8 - 35.9	0.1	30	7.93	7.81 - 8.12	0.06	30	2284	2192 - 2421	52
	PD-14.7	108	14.7	14.6 - 14.9	0.1	30	35.9	35.8 - 35.9	0.1	30	7.94	7.75 - 8.16	0.07	30	2288	2184 - 2421	46
	PD-15.7	59	15.7	15.4 - 15.9	0.1	15	35.9	35.8 - 36.0	0.1	15	7.91	7.83 - 7.97	0.04	15	2248	2194 - 2285	24
	PD-13.0	43	13.0	12.8 - 13.2	0.1	10	35.9	35.8 - 36.0	0.1	10	7.91	7.81 - 7.97	0.05	9	2246	2224 - 2273	15
	Tank200-I									35	7.95	7.88 - 8.03	0.03	64	2266	2199 - 2317	23
	Tank200-II									13	7.95	7.88 - 8.00	0.03	49	2484	2464 - 2514	14

Table 4.2: Summary of the physico-chemical conditions for each system (CSI, CSII and PD) and in the 200-litres tanks (Tank200-I and Tank200-II): average, range and standard deviation. The salinity data were standardised using the values of Tank200-I or Tank200-II whereas pH and alkalinity values are raw data. The values presented for CSI correspond to the measurements performed at both sampling locations in the systems (at the outlets of the 20-litres tank and of the 3 experimental bottles).

4.1.1. Temperature

Figure 4.8 presents the average daily value of the temperature measurements inside the incubators. We decided to present the data as daily averages because the raw data (1 measure every 10 min) recorded the short peaks of higher air temperature inside the incubators caused by the opening of the door (e.g. for water sampling or simple checks). These temperature data do not correspond to the temperature of the water inside the incubator since during short-term openings, the experimental water will not have the time to warm up. The average values take into account all the raw data but allow to illustrate the events that could have an impact on the geochemistry of foraminiferal tests. PD-7.9, PD-10.2, PD-12.7 and PD-14.7 were respectively in the same incubators as CSI-7.9, CSI-10.1, CSI-12.7 and CS-14.7 and therefore their temperature records are presented together in Figure 4.8.

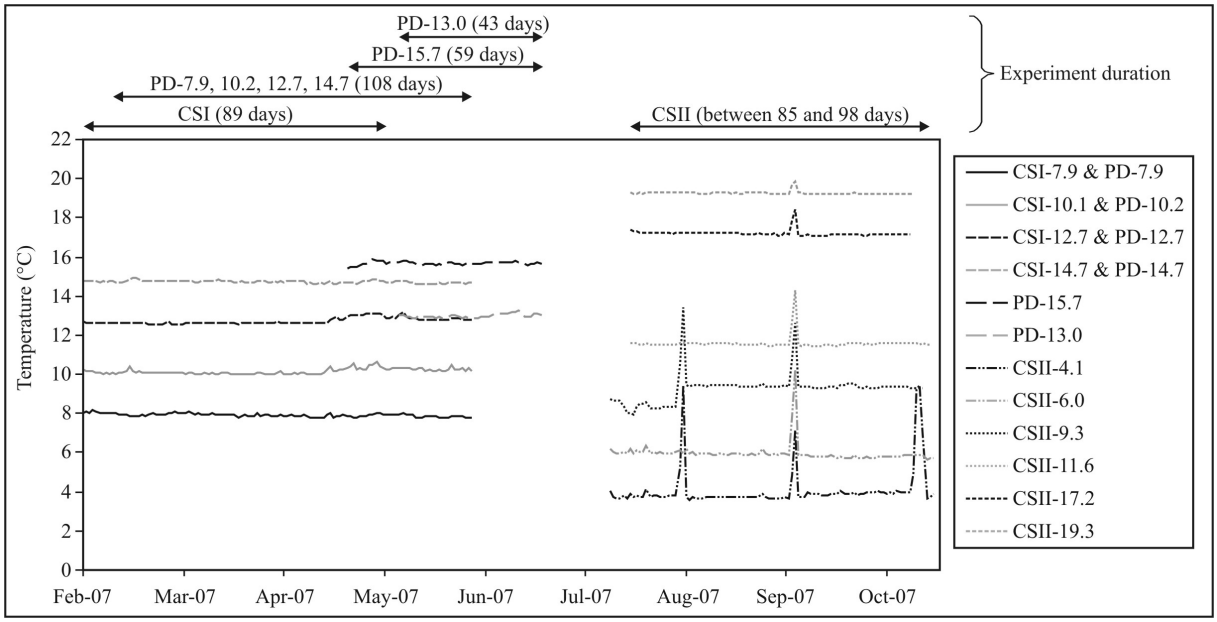


Figure 4.8: Temperature variability of the systems plotted as a function of the date of the experiments.

The average daily temperatures were very stable in the CSI and PD experiments with standard deviations lower than 0.2°C (Table 4.2). Temperatures recorded for CSII experiments are also stable but there are several high temperature events recorded during the experimental period. On the 5th of September 2007, the peak recorded in all the systems corresponds to an electricity cut. Note that peaks are more important in the coolest incubators. This can probably be explained by the high room temperature (about 25°C) at this time.

The other temperature peaks, concerning particularly the incubators fixed at 4 and 9°C, can probably be explained by the fact that the door was not properly closed between two observations. For the incubator at 9°C, a problem occurred on the 2nd of August 2007 (t=20 days) when the temperature displayed by the incubator changed from 8 (fixed temperature) to 9°C without a clear explanation. However, after this 1°C shift, the incubator temperature remained very stable until the end of the experiment.

4.1.2. Salinity

Precise salinity measurements were performed at the INSU laboratory of Roscoff for the seawater used in CSI, at the beginning and at the end of the experiment. Seawater of Tank200-I (condition at the beginning of CSI experiments) had a salinity of 35.840‰ and seawater of the 20-litres tanks of CSI-10.1, CSI-12.7 and CSI-14.7 at the end of the experiment gave an average value of 35.679±0.089‰. From these precise data, we can infer that the systems did not undergo evaporation.

In Figure 4.9 and Table 4.2, salinity measurements performed with the conductimeter have been standardised with the salinity of the 200-l tanks (Tank200-I for CSI and PD, and Tank200-II for CSII) to remove the variability of the measurement device.

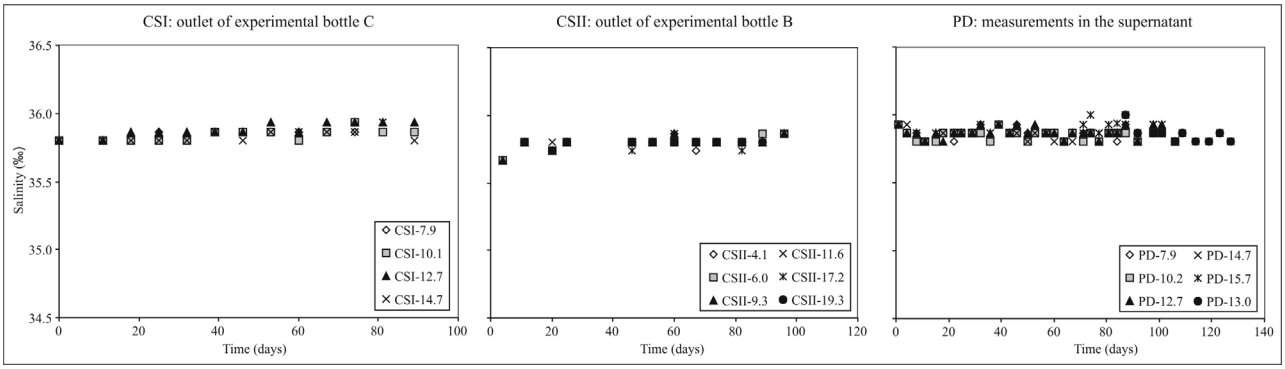


Figure 4.9: Salinity variability of the systems. The data presented have been standardised using the data of Tank200-I or Tank200-II. Only salinity values at the outlet of the 3 experimental bottles are presented for CSI.

For CSI, comparison of the salinity measured in the seawater before entering the experimental bottles (at the outlet of the 20-litres tank; average salinity for the 4 systems: 35.8 ± 0.1‰) and at the outlet of the third bottle C (average salinity for the 4 systems: 35.8 ± 0.1‰) shows no difference. Therefore, we can infer that the salinity conditions of the seawater inside the 3 experimental bottles are similar. No major difference was recorded either between the average

salinity of the supernatant (35.8 ± 0.1‰, raw data) and Tank200-I (35.7 ± 0.1‰, raw data) for the PD experiments. Maximum standard deviation is ± 0.1‰ in all the experiments. We can therefore conclude that salinity conditions have been stable during the experiments of the three systems (CSI, CSII and PD) (Figure 4.9 and Table 4.2).

4.1.3. pH

For CSI, the conditions are stable and similar at the outlet of the 20-litres tank (average pH for the 4 systems: 7.95 ± 0.04) and at the outlet of experimental bottle C (average pH for the 4 systems: 7.97 ± 0.04). Considering the precision of the measurements, there is no evolution of the pH when flowing through the 3 experimental bottles.

The average pH values are very similar in all the systems (between 7.91 and 7.98) except for CSII where the average values are around 7.8 (Table 4.2). This is because the pH decreased during the CSII experiments, with a maximum drop of 0.3 between the beginning and the end of the experiments (Figure 4.10). This event happened in all CSII systems (CSII-4.1, CSII-6.0, CSII-9.3, CSII-11.6, CSII-17.2 and CSII-19.3) but was not observed in Tank200-II (Figure 4.10). This point will be discussed later (see § 5.1.2.).

For PD-7.9, PD-10.2, PD-12.7 and PD-14.7, the pH of the water was higher after one day (t=1; 8.13 in average in the 4 Petri dishes) than at its introduction one day earlier (t=0; 7.93 for Tank200-I). The pH values in the supernatant of these 4 Petri dishes decreased in 8 days to reach a “normal” value of approximately 7.95; this value is similar to the one of Tank200-I used to fill up the Petri dishes (Figure 4.10). This phenomenon was not observed at the beginning of PD-15.7 and PD-13.0, started at t=70 and t=86 days, respectively.

Some pH variations observed in the systems seem to be the result of temporal variations of the measuring instrument, to the device since the same variations are observed in Tank200-I and/or II and in the system (Figure 4.10, arrows).

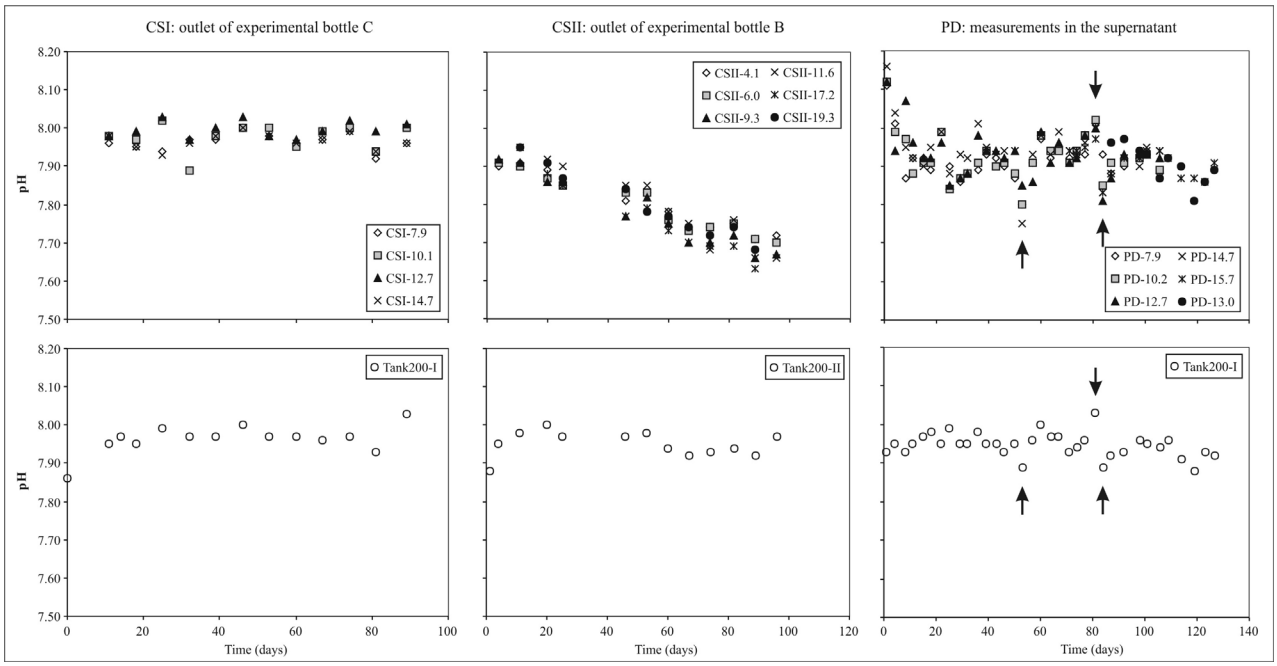


Figure 4.10: pH variability of the systems (raw values). pH measurements performed at the same date in the systems (upper panels) and in the 200-l tanks (lower panels) are both presented. Variations that are probably caused by the measuring equipment (observed in a system and in the respective 200-l tank at the same time) are indicated by arrows. Only pH values at the outlet of the 3 experimental bottles are presented for CSI.

4.1.4. Alkalinity

Considering the precision of the measurements (2% and 0.7% for the manual titrations and Methrom titrations, respectively), conditions are stable in the experiments CSI, CSII, PD-15.7 and PD-13.0 (Table 4.2). Just as for the pH, we noticed higher alkalinity values at the start of PD-7.9, PD-10.2, PD-12.7 and PD-14.7 (in average 2417 µmol.l^{-1}) in comparison to the alkalinity of the seawater from Tank200-I (in average 2294 µmol.l^{-1}) used to fill up the Petri dishes (Figure 4.11). At t=11 days, the alkalinity in the supernatant of the Petri dishes became similar to the alkalinity measured in the Tank200-I. From there on, the alkalinity measurements remained stable until the end of the experiment. No increase of the alkalinity was recorded at the start of experiments PD-15.7 and PD-13.0. The decrease in alkalinity observed around t=80-90 days could be partly due to a high variation in the replicate measurements at these dates (standard deviations of 40 µmol.l^{-1} compared to ~10 µmol.l^{-1} the rest of the time for replicate measurements in Tank200-I; see Figure 4.11, lower panel on the right).

Average alkalinity values are similar for CSI and PD (2283 µmol.l^{-1}), but the average values are higher for CSII (2488 µmol.l^{-1}) by around 200 µmol.l^{-1}. These higher values reflect the higher alkalinity in Tank200-II.

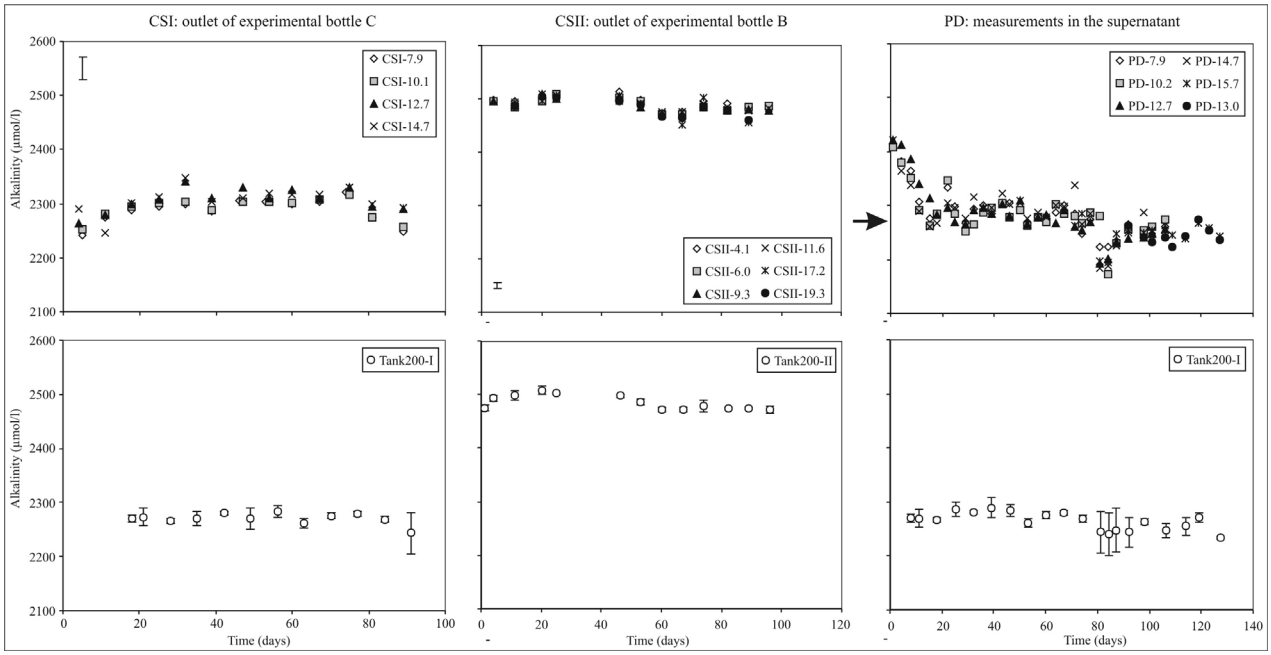


Figure 4.11: Alkalinity variability of the systems (raw values). Alkalinity measurements performed at the same date in the systems (upper panels) and in the 200-l tanks (lower panels) are presented together.

4.1.5. DIC

The measurements of DIC were not precise enough to be useful for monitoring the variability of this parameter. Therefore we calculated the DIC values using the program CO_2sys with pH and alkalinity values as input data. The conditions in CSI are stable whereas DIC increases in CSII during the experiments by about 100 µmol.kg^{-1} (Figure 4.12). For PD, we can notice an increase of DIC values at the beginning of the experiments PD-7.9, PD-10.2, PD-12.7 (no increase for PD-14.7). This peak occurs after the peaks of alkalinity and pH in the same Petri dishes.

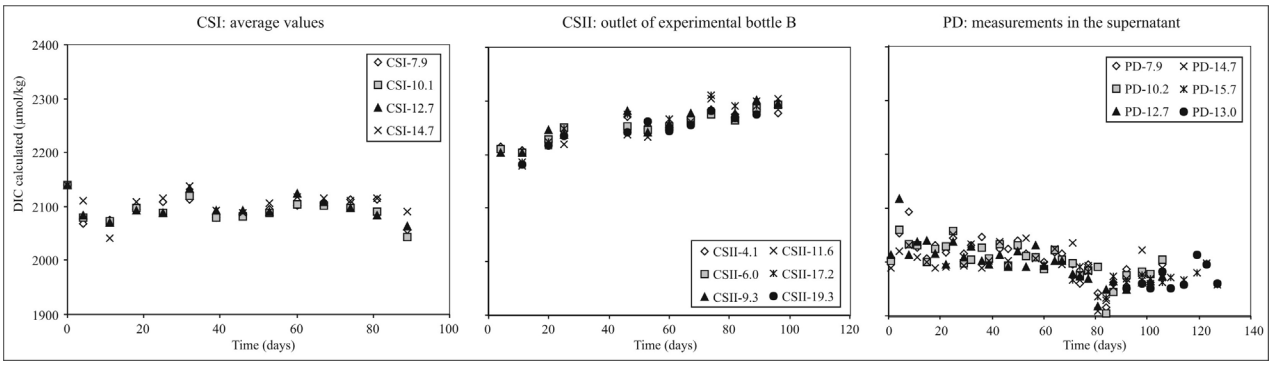


Figure 4.12: Calculated DIC values of the systems (using the CO_2sys program with pH and alkalinity values as input data).

4.2. Foraminiferal culture results

Part of the marked adult specimens of *Bulimina marginata* added at the beginning of the experiments (between 9 to 70%) calcified new chambers in our experiments (Table 4.3-a). However, no specimens calcified more than 3 new chambers. Specimens of *Hyalinea balthica* were also successful in calcifying new chambers. For all the experiments in which we introduced *H. balthica*, more than 23% of the specimens calcified new chambers within the time of the experiment (Table 4.3-b). Moreover, this species was able to calcify from 2 to 8 new chambers (4 chambers in average), much more than *B. marginata*.

In all the experiments of all systems, we obtained reproduction of *Bulimina marginata*. Unfortunately, no other species than *Bulimina marginata* reproduced in our experiments. In the experiments PD-7.9, PD-10.2, PD-12.7, PD-14.7 and PD-13.0, only adults of *B. marginata* f. *marginata* were added at the beginning of the experiments; and only juveniles showing a typical morphology of the *marginata* morphotype were produced. In the other experiments containing a mix of adults of both morphotypes (*marginata* and *aculeata*), either only specimens of *marginata* morphotype were produced (CSI-7.9, CSI-10.1, CSII-4.1, CSII-6.0, CSII-9.3 and CSII-11.6) or specimens of both morphotypes were obtained (CSI-12.7, CSI-14.7, CSII-17.2, CSII-19.3 and PD-15.7). Specimens of *aculeata* morphotypes were only produced when the temperature was equal or higher than 13°C, and when adults of this morphotype were incubated in the experiment.

		CSI				CSII						PD					
		CSI-7.9	**CSI-10.1**	**CSI-12.7**	**CSI-14.7**	**CSII-4.1**	**CSII-6.0**	**CSII-9.3**	**CSII-11.6**	**CSII-17.2**	**CSII-19.3**	**PD-7.9**	**PD-10.2**	**PD-12.7**	**PD-14.7**	**PD-15.7**	**PD-13.0**
	Temperature (°C)	**7.9**	**10.1**	**12.7**	**14.7**	**4.1**	**6.0**	**9.3**	**11.6**	**17.2**	**19.3**	**7.9**	**10.2**	**12.7**	**14.7**	**15.7**	**13.0**
(a)	Nb of *B. marginata* adults that calcified new chambers (*quantity introduced*)	25 (*211*)	30 (*200*)	26 (*213*)	34 (*202*)	43 (*100*)	62 (*100*)	53 (*100*)	34 (*150*)	14 (*150*)	14 (*150*)	21 (*30*)	14 (*30*)	16 (*30*)	8 (*30*)	n.d.	n.d.
(b)	Nb of *H. balthica* adults that calcified new chambers (*quantity introduced*)	9 (*30*)	9 (*30*)	10 (*30*)	7 (*30*)	-	-	2 (*3*)	4 (4)	3 (4)	3 (4)	-	-	-	-	-	-
(c)	Total number of *B. marginata* juveniles produced	**304**	**777**	**790**	**775**	**110**	**261**	**2461**	**567**	**590**	**282**	**402**	**593**	**585**	**445**	**214**	**890**
(d)	% of *B. marginata* juveniles produced ≥150 µm	13	42	30	27	0	0	9	37	20	22	20	16	15	10	53	25
(e)	% marked juveniles of *B. marginata* that calcified new chambers	-	-	-	-	-	-	-	-	-	-	27	42	47	43	-	-

Table 4.3: Results of the foraminiferal cultures in the systems. (a) and (b) present respectively the number of adult specimens of B. marginata *and the number of* H. balthica *that calcified new chambers in the controlled systems (the number in parenthesis indicates the total number of adult specimens introduced); (c) and (d) present the total number of juveniles of* Bulimina marginata *produced in the systems as well as the number of produced specimens that reached a size of ≥ 150 µm (strategy 1); and (e) presents the number of marked juveniles added in the experiments that calcified new chambers in the controlled conditions (strategy 2).*

The quantity of juveniles produced per systems (CSI, CSII and PD) seems to be dependant on temperature (Figure 4.13 and Table 4.3-c). The number of juveniles produced tends to be higher at intermediate temperatures, between 9 and 15°C. Especially in experiment CSII-9.3 (9.3°C), a very high number of juveniles (2461 specimens) was produced. However, only 9% of these juveniles grew until 150 µm or more, and 62% stayed smaller than 100 µm (Table 4.3-d). Under more elevated temperatures, a much larger part of the juveniles grew to an adult size (<150 µm).

We also observed that at the lowest temperatures (CSII-4.1 and CSII-6.0 at 4.1 and 6.0°C, respectively), none of the produced juveniles attained a length larger than 150 µm. In all other experiments, a 150 µm size was reached by 9 to 53% of the produced juveniles (Table 4.3-d).

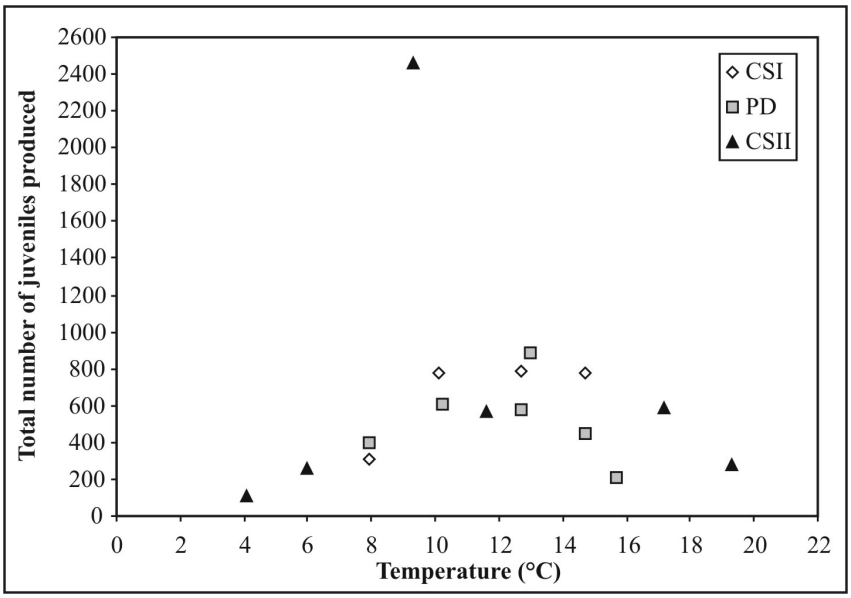


Figure 4.13: Total number of juveniles born in the different systems according to the temperature of the experiments.

In PD-7.9, PD-10.2, PD-12.7 and PD-14.7, we also applied strategy 2 which consisted in the introduction of small (2 to 3 chambers) marked juvenile specimens of *Bulimina marginata* in the culture jars in order to obtain growth of these juveniles until an adult size (see § 3.1.1. and Figure 4.1). Between 27 and 47% of the 250 marked juvenile specimens of *Bulimina marginata* added at the start of the experiments calcified large number of new chambers in the controlled conditions (Table 4.3-e). Specimens that calcified new chambers reached an average length of 205 ± 30 µm. The average length of the pool of juveniles added at the start of the experiment was 94 ± 10 µm. The increase of 110 µm in length corresponds to an average volume of calcite increased by 4.75. This means that 80% of the final foraminiferal shell volume was constituted of calcite formed in controlled conditions.

5. DISCUSSION

5.1. Stability of the systems

In our experiments, we studied the impact of a single parameter, temperature, on the $\delta^{18}O$ composition of the deep-sea benthic foraminiferal shell. The oxygen isotopic composition of the foraminiferal shell depends mainly on temperature, salinity and $\delta^{18}O_{seawater}$. It was essential to maintain these three parameters stable during the experiments. However, also parameters related to carbonate chemistry (pH, alkalinity, pCO_2…) have an influence on the

isotopic composition of carbonate (Spero *et al*., 1997; Zeebe, 1999), and preferably should be maintained stable. Each system lasted for 3 months to 3.5 months, except for PD-15.7 and PD-13.0. During these long-term experiments, we aimed to keep our systems as stable as possible.

5.1.1. Temperature, salinity and $\delta^{18}O_{seawater}$

In all systems (CSI, CSII and PD), we succeeded to avoid any evaporation of seawater, and increases of salinity (Figure 4.9). The salinity variations observed in our experiments were not significant considering the precision of the measuring instrument (standard deviation of each experiment ± 0.1‰; Table 4.2).

The temperature measurements show also very stable conditions except for some periods in the CSII experiments where short peaks of high temperatures occurred (Figure 4.8). For CSII-6.0, CSII-11.6, CSII-17.2 and CSII-19.3, the only high temperature event (electricity cut) corresponds to 2% of the total duration of each experiment, whereas for CSII-4.1, higher temperatures represent 8% of the total experimental duration. We think that there is a low probability that an important proportion of the shells calcified because of the briefness of these events. Furthermore, the temperature recorded during these experiments was the temperature inside the incubators and not the temperature of the experimental seawater itself. We would expect the temperature of approximately 20 litres of seawater (in the case of CSII) to increase slowly compared to the air temperature. Although these temperature changes could possibly have an impact on the $\delta^{18}O$ composition of foraminiferal shell, we assume that it is negligible. The case of CSII-9.3 is more problematic since the temperature of the incubator increased by 1°C at 20 days after the beginning of the experiment. However, Barras *et al*. (submitted) observed that adults of *Bulimina marginata* can reproduce in the presence of fresh *phaeodactylum* within 11 to 15 days. It is therefore possible that adult specimens in CSII-9.3 reproduced before the temperature shift, but that the juveniles did not have the time to calcify many chambers. We can consequently expect that this 1°C shift will have a small impact on the isotopic composition of the shells.

The $\delta^{18}O$ of the seawater depends only on the stability in time of the 200-litres tanks and on the evaporation during the experiments. Both processes are well controlled and therefore the $\delta^{18}O_{seawater}$ remained certainly constant during the experiments. The measurements carried out in Gif-sur-Yvette should confirm the stability of the oxygen isotopic composition of the seawater in the systems. Therefore, the $\delta^{18}O$ composition of foraminifera depends on the

variations on temperature. An increase of 1°C would result in a decrease of around 0.25‰ of the $\delta^{18}O$ composition of foraminiferal shell.

5.1.2. Carbonate chemistry of the seawater

During our experiments, we equally measured pH, alkalinity and estimated DIC (calculated with the CO_2sys program using alkalinity and pH data as input values), in order to survey the carbonate chemistry of the seawater. It is particularly difficult to maintain stable the carbonate chemistry of the seawater, because it depends on several processes such as respiration, photosynthesis, precipitation and dissolution of $CaCO_3$. The different carbonate species are in equilibrium in the seawater. The equilibrium between $CO_{2(aq)}$ (which is partially hydrated into carbonic acid, H_2CO_3), bicarbonate and carbonate ions can be described by the following equation:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2\ H^+ + CO_3^{2-} \qquad (1)$$

The relative proportion of the different species depends on the pH of the seawater (Figure 4.14).

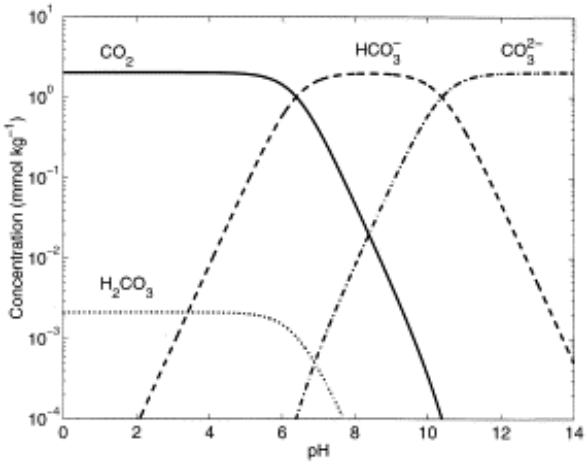


Figure 4.14: The concentration of dissolved carbonate species as a function of pH: CO_2 (solid line), H_2CO_3 (dotted line), HCO_3^- (dashed line), and CO_3^{2-} (dotted–dashed line). The values shown correspond to fresh water conditions (T = 19°C, ΣCO_2 ≈2 mmol.kg^{-1}) (Zeebe, 1999).

In the presence of calcareous organisms, precipitation (equation 2) and dissolution of $CaCO_3$ (equation 3) have an impact on the carbonate chemistry of the seawater:

$$Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \qquad (2)$$

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2\ HCO_3^- \qquad (3)$$

The precipitation of calcium carbonate uses bicarbonate ions and releases CO_2. Therefore, in the case of calcite production, pH, DIC but also alkalinity will decrease. In fact, the carbonate alkalinity corresponds to $[HCO_3^-] + [CO_3^{2-}]$ so that the transformation of HCO_3^- into CO_2 decreases the alkalinity of the system. The dissolution of $CaCO_3$ has an opposite effect with an increase of pH, DIC and alkalinity of the seawater.

Respiration or oxidation of the organic matter (equation 4) and photosynthesis (equation 5) have opposite effects on the carbonate chemistry as shown by their equations:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \quad (4)$$

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 \quad (5)$$

Since CO_2 is released into the system during respiration, this process is decreasing the pH of the seawater and increases the DIC. On the contrary, CO_2 uptake during photosynthesis causes more alkaline conditions and a decrease of DIC. Both reactions have no influence on alkalinity but the proportions of the carbonate species change: the CO_3^{2-} concentration increases in the case of photosynthesis and decreases during respiration. It is important to note that in our culture conditions, we should not observe any photosynthesis effects, since deep-sea benthic foraminifera are non-symbiont bearing heterotrophic organisms and our experiments took place in the dark.

Even if the carbonate concentration in seawater is not the main parameter controlling the oxygen isotopic fractionation, it has been demonstrated that there is a relation between the $\delta^{18}O$ composition of planktonic foraminifera and the concentration in CO_3^{2-} and therefore also with pH (Spero *et al*., 1997). Spero *et al*. (1997) established in culture experiments that the $\delta^{18}O$ of *Orbulina universa* decreases by around 0.002‰ per $\mu mol.kg^{-1}$ increase in $[CO_3^{2-}]$ under dark conditions. This slope was doubled for *Globigerina bulloides* which is a non-symbiont bearing foraminifer (0.004‰/$\mu mol.kg^{-1}$). Zeebe (1999) proposed a possible explanation for this pH dependency of foraminiferal oxygen isotopic composition. The relative proportion of the different carbonate species (equation 1) depends on pH and they have different isotopic compositions: the $\delta^{18}O$ of HCO_3^- is heavier than that of CO_3^{2-}. Therefore, the $\delta^{18}O$ of the calcite that calcifies in equilibrium with ambient seawater depends on the relative concentrations of the carbonate species, more specifically, on pH and $[CO_3^{2-}]$ (Zeebe, 1999).

For CSI experiments, pH, alkalinity and DIC (calculated) values were reasonably stable. The maximum standard deviation for one system was ± 0.03 for the pH, ± 34 $\mu mol.l^{-1}$ for the

alkalinity and ± 24 µmol.kg^{-1} for the DIC. These variations are all inferior to the precision of the measurements (DIC not considered).

For experiments PD-7.9, PD-10.2, PD-12.7 and PD-14.7 (in comparison with the 200-litres tank), we observed an increase of pH and alkalinity of the seawater at the beginning of the experiments. The calculated DIC (Figure 4.12) indicates a small increase which is of shorter duration than the shift in pH and alkalinity conditions (Figures 4.10 and 4.11).
On Figures 4.10 and 4.11, we can notice that the increases of pH and alkalinity occurred in the first days of the experiment. We renewed the seawater every 3-4 days with water from Tank200-I so that the pH and alkalinity values in the Petri dishes decreased until normal values, similar to the conditions in the Tank200-I, occurred after about 2 weeks. We think that the increase in pH, alkalinity and DIC at the beginning of the experiment can have been caused by the partial dissolution of the calcium carbonate of the incubated foraminifera. We can see from equation (3) that the dissolution of one mole of $CaCO_3$ would lead to an increase of two moles in total alkalinity and one mole in DIC, explaining the more important shift in alkalinity than in DIC. However, we observed no signs of dissolution on the foraminiferal shells in any of the Petri dishes. All foraminiferal tests were intact (naked-eye observation) in the first weeks of the experiments. It is also strange that this increase in pH and alkalinity was not observed in the experiments PD-15.7 and PD-13.0, which started later (at t=70 and t=86 days, respectively). The main difference between these two groups of Petri dishes was that, in PD-7.9 to PD-14.7, a high number of marked juveniles of *B. marginata* were introduced (250 specimens) to obtain growth of these specimens (strategy 2). That was not the case for PD-15.7 and PD-13.0. In PD-7.9 to PD-14.7, the introduction of many juveniles in the new medium may have led to dissolution of the surface of the small shells and consequently to the increase of pH and alkalinity. However, this putative dissolution event has not been seen under the stereomicroscope. In all cases, since all conditions became normal after about 2 weeks, we conclude that the process responsible for these shifts disappeared rapidly after the introduction of the foraminifera.

The foraminiferal faunas were observed every time the water of the Petri dishes was changed (every 3 to 4 days). We know therefore when reproductions took place. The first reproduction of adult specimens of *Bulimina marginata* (strategy 1) occurred in PD-12.7 and PD-14.7 (at 12.7 and 14.7°C, respectively) 11 days after the start of the experiments. Considering the marked juveniles added to the Petri dishes (strategy 2), we first observed newly formed

calcite 8 days after the start of the experiments. This means that only a low quantity of calcite was formed during the high pH and alkalinity conditions (until t=15 days). We can therefore assume that neither the juveniles born in the experiments, nor the marked juveniles that calcified new chambers have been significantly influenced by this shift in alkalinity and pH. The majority of the specimen shells calcified under stable pH and alkalinity conditions, recorded from the 15th day until the end of the experiments. Consequently, we think that this event should have only a minor impact on $\delta^{18}O$ of foraminiferal shells.

For CSII, we observed a decrease of the pH during the experiments (Figure 4.10) whereas the alkalinity remained stable considering the precision of the measurements (Figure 4.11). Logically, the DIC values determined with the CO_2sys program show a slight increase.
The mechanism responsible for the decrease in pH and the increase in DIC in CSII is most probably the mineralisation of organic matter (i.e. diatoms introduced in the experimental bottles). According to the respiration equation (5), the mineralisation of organic matter would increase the CO_2 concentration in seawater and decrease the pH. In an open system, the excess CO_2 would diffuse to the atmosphere, allowing to reach an equilibrium. However, in a gas tight system, it would be forced to remain as $CO_{2(aq)}$. The main difference in the protocol between CSI, where we did not observe a decrease in pH, and CSII was the type of tubing used to connect the tanks to the experimental bottles. The silicon tubing used for CSI is almost 200 times more permeable than the Tygon® tubing used for CSII. We suspect that the silicon tubing avoids the evaporation of seawater but gases are still exchanged between the system and the atmosphere. Apparently, this gas exchange is considerably reduced with Tygon® tubing. We suppose that the first group of closed systems (CSI) was in reality a semi-closed system where dissolved CO_2 generated by respiration was equilibrated with atmospheric CO_2. The second group of closed systems (CSII) was tight enough to maintain the $CO_{2(aq)}$ in the seawater so that the pH decreased and DIC increased whereas alkalinity remained stable. However, the drop in pH could also (partially) result from precipitation of calcium carbonate in the system (equation 2). This second possibility is less probable, because in the case of calcification, the decrease in pH should be accompanied by a decrease in alkalinity and DIC.
As we mentioned before, the pH and/or $[CO_3^{2-}]$ of the seawater seems to have an influence on the $\delta^{18}O$ composition of foraminifera (Spero *et al*., 1997; Zeebe 1999). Zeebe (1999) estimated that an increase of seawater pH of 0.2-0.3 units would cause a decrease of 0.22-0.33‰ in $\delta^{18}O$. Therefore, the decrease of pH recorded in CSII (from 7.9 to 7.6

approximately, Table 4.2) could have an impact of 0.3‰ on the $\delta^{18}O$ composition of the foraminiferal shells. With the CO_2sys program, $[CO_3^{2-}]$ could also be estimated from alkalinity and pH, and the shift in $[CO_3^{2-}]$ between the start and the end of the experiment was 80 $\mu mol.kg^{-1}$ maximum. If we use the equation of Spero *et al*. (1997) for *G. bulloides* (non-symbotic planktonic foraminifera, 0.004‰ per $\mu mol.kg^{-1}$) to calculate the maximum possible impact on $\delta^{18}O$ foraminiferal calcite, we arrive at again a maximum depletion of about 0.3‰. If the totality of the calcite had been formed at the end of the 3 months of experiments, this $\delta^{18}O$ depletion should be noticeable when comparing foraminifera calcified in CSII with foraminifera calcified in CSI and PD (where pH was stable around 7.9). Considering that adult specimens of *B. marginata* are reproducing on average within 15 days after the addition of *Phaeodactylum* (Barras *et al*., submitted), we can suppose that the shells were calcified all over the experimental duration. Since the pH decrease appeared progressively in the systems, we could expect that the impact will be at most 0.15‰. It should be kept in mind, however, that the influence of the carbonate chemistry was reported to be smaller on the benthic foraminifer *Oridorsalis umbonatus* sampled from core tops (Rathmann and Kuhnert, 2008). The isotopic measurements of the foramniferal shells calcified in this condition are presented and further discussed in Chapter 5.

The absolute values of alkalinity of both seawater sets, Tank200-I (2266 $\mu mol.l^{-1}$) and Tank200-II (2484 $\mu mol.l^{-1}$), present a difference of 218 $\mu mol.l^{-1}$ in average. The total alkalinity in the North Atlantic Ocean varies between 2280 and 2350 µeq.kg-1 between surface water and 6 km water depth (Takahashi *et al*., 1981). The difference observed between both 200-l tanks is higher than the range measured in the water column of the Atlantic Ocean. This difference can therefore not be explained by the fact that Tank200-I was superficial seawater and Tank200-II has been sampled at 100 m water depth. The difference in the protocols used to titrate the alkalinity between the 2 systems (CSI = manual titrations, CSII = automated titrations) can probably explain part of this difference. On the one hand, the 0.5 HCl used for the manual titrations was not certified but titrated to determine its precise concentration. On the other hand, the dilution for the manual method was 10%, due to the addition of HCl in the seawater sample, whereas it was only 4% for the automatical method. It is probable that this dilution effect is responsible for a large part of the lower absolute value of alkalinity found with the manual method.

5.1.3. Comparison with published culture systems

The closed systems performed in this study were inspired by the system described by Hintz *et al*. (2004). They studied the isotopic and trace metal composition of the shells of culture benthic foraminifera. Their system consisted of a much higher quantity of water (1600 litres tanks) which was open to the atmosphere (Hintz *et al*., 2004). Therefore they did not observe a decrease of pH in their systems or an increase in salinity. The stability of temperature and salinity (± 0.3-0.5°C for temperature and ± 0.3-0.2‰ for salinity, depending on the experiment) were comparable to those obtained in our experiments (± 0.1-0.7°C for temperature depending on the experiment, and ± 0.1‰ for salinity). Hintz *et al*. (2004) succeeded to keep very stable conditions concerning the carbonate chemistry of the seawater (maximum standard deviations of ±0.027 for pH, ±5 $\mu eq.kg^{-1}$ for alkalinity and ±6 $\mu mol.kg^{-1}$ for DIC). The standard deviations observed during their long-term experiments (3 to 8 months) are smaller than the variations observed in our experiments for pH and alkalinity. They performed high precision measurements for these parameters which required high sample volumes. This is of course only possible if large volumes of seawater are used for the system. McCorkle *et al*. (2004) reached the same stability in their experiments with benthic foraminifera using a similar type of system. In their laboratory experiments, both Hintz *et al*. (2004) and McCorkle *et al*. (2004) used a mixture of natural and artificial seawater. Their absolute values for alkalinity and DIC were much higher than for natural seawater (between 2959 and 3176 $\mu eq.kg^{-1}$ for alkalinity and between 2669 and 2885 $\mu mol.kg^{-1}$ for DIC). Therefore, the isotopic composition of the foraminiferal calcite formed in these conditions can not be compared to the one of *in situ* specimens. In our opinion, it seems preferable to use natural microfiltrated seawater for culture experiments but the carbonate chemistry of seawater should be measured more precisely than in our study. The major problem in the system developed by Hintz *et al*. (2004) is that, because it is widely opened to the atmosphere, they recorded in the $\delta^{13}C_{DIC}$ composition of the seawater the seasonal variation of $\delta^{13}C$ in the atmosphere. Therefore, in the case of the study of $\delta^{13}C$ composition of foraminiferal calcite in culture experiments, it appears to be essential to use a system that is closed to the atmosphere or to reduce the experimental duration.

Toyofuku *et al*. (2000) performed culture experiments in Petri dishes to investigate the impact of temperature on the Mg/Ca content of shallow benthic foraminiferal shells. They changed the water every day, and observed variations smaller than 1‰ in salinity, whereas the temperature of their different experiments varied by ±0.1 to 1.6°C.

All laboratory cultures performed with planktonic foraminifera present the advantage to be short-term experiments achieved within 6 to 15 days (e.g. Erez and Luz, 1983; Spero and Lea, 1996; Spero *et al*., 1997; Bijma *et al*., 1998; Russell *et al*., 2004). When data about the different parameters measured are given, conditions appear to be as stable as in our systems. For example, Spero *et al*. (1997) recorded variability of ±0.2°C for temperature and of ±15-80 $\mu eq.kg^{-1}$ for alkalinity which is of the same order of magnitude as in our systems.
In conclusion, both in the first group of closed systems (CSI) and Petri dish systems described in this paper, physico-chemical conditions were just as stable as in the systems described in the literature, considering the precision of our measurements. Only CSII systems, in which the pH decreased during the experiment, were less stable, probably because the systems were gas tight and avoided equilibration with the atmosphere of the CO_2 released by the mineralisation of the organic matter.

5.2. Foraminiferal culture results

- *Bulimina marginata* – strategy 1 (introduction of adult to obtain reproduction and growth of the juveniles produced):

Adults of *B. marginata* reproduced successfully over a large range of temperature (4 to 19°C). The number of juveniles of *Bulimina* produced was higher at temperatures between 9 and 15°C. Barras *et al*. (submitted) observed that the total number of juveniles produced by this species was more important at intermediate temperature (between 8 and 12°C), whatever the diet (*Phaeodactylum* or *Chlorella*). It seems therefore that there is an optimal range of temperature within which the production of juveniles is increased. We can suppose that beyond a certain critical threshold value, very high or very low temperatures, *Bulimina* is no longer able to reproduce.
The maximum size of the juveniles of *Bulimina marginata* produced in the culture experiments was about 300 µm, which is smaller than the average size of the adults at the beginning of the experiments. At the lowest temperatures tested in our experiments (4 and 6°C), we succeeded to obtain reproduction of the adults of *Bulimina marginata* but the juveniles did only show a limited growth. However, the mass of calcite produced was sufficient to be able to measure the $\delta^{18}O$ of the foraminiferal shells produced in our controlled conditions for all the experiments. Of course, the bigger the juveniles are, the better it is, because (1) less specimens are required to obtain a measurement and replicate measurements become possible (same species, same size, same temperature, same experimental bottle or

Petri dish) and (2) we can investigate the ontogenic effect on the oxygen isotopic composition (see Chapter 5). Longer-lasting experiments will perhaps allow to obtain bigger specimens, especially for the coldest temperatures tested.

- *Bulimina marginata* – strategy 2 (introduction of small labelled juveniles to obtain growth):

The use of marked juveniles gave interesting results. On the average, 40% of the marked juveniles introduced in our experiments succeeded to calcify new chambers, until an average size of 205 µm was reached. The proportion of newly calcified material under the controlled conditions was approximately 80% of the total volume of calcite. We could suppose therefore that the $\delta^{18}O$ signal recorded for these specimens at the end of the experiment should be strongly dominated by the calcite formed in the controlled conditions. However, the $\delta^{18}O$ values showed that the 20% of calcite formed prior to controlled conditions has an influence on the δ18O composition of the entire shells (see Chapter 5). In order to avoid any possible bias, it should be possible to separate the youngest chambers calcified before the beginning of the experiments by laser microdissection. However, such a method is very time consuming, and will inevitably lead to the loss of many tests.

- *Hyalinea balthica* – strategy 1:

As far as we know, *Hyalinea balthica* was not used in laboratory experiments before. This species did not succeed to reproduce in the experiments presented in this paper. However, reproduction of *H. balthica* was observed in preliminary experiments. We think that the particular conditions necessary to stimulate the reproduction of this species were not present in our culture setup. Nevertheless, in all the experiments where adults of this species were introduced (from 8 to 19°C in CSI and CSII), newly calcified chambers were obtained. Moreover, specimens were calcifying a relatively high number of chambers (up to 8 chambers) in the time of the experiment (around 3 months). Therefore *Hyalinea balthica* seems to be a very interesting species for measurements using laser ablation techniques, e.g. investigation of trace metal elements in a single chamber (Eggins *et al*., 2003; Reichart *et al*., 2003; Rathmann and Kuhnert, 2008). We suppose that the fresh diatoms added in our experiments stimulated the calcification of new chambers. In fact, this preferentially shallow infaunal species (Schmiedl *et al*., 2000) has been observed in areas where high quantity of fresh labile organic matter was deposited (Schmiedl *et al*., 2000; Bartels-Jónsdóttir *et al*., 2006).

In our experiments, we obtained growth of adult specimens between 8 and 19°C. According to Murray (1991), this species occurs alive at temperatures ranging from 9 to 13°C. An inventory of the collections BIAF laboratory showed that living specimens are found between 8 and 13°C (Diz Ferreiro, pers. comm.).
In future work, it would be interesting to perform additional experiments with this species to find out the optimal conditions to obtain reproduction and growth of *H. balthica* specimens.

5.3. Comparison of the two systems

On the basis of our results, we can conclude that with both the closed system and the Petri dish system, it is possible to maintain stable conditions during long-time experiments in a cheap and easy way. In the case of the closed system, the choice of silicon tubing rather than Tygon® should be preferred if the aim is to avoid evaporation of the seawater. If a gas tight system is required, the quantity of food added in the experiment should be reduced in order to avoid acidification of the seawater. For the PD system, it may be better to avoid the presence of too many specimens in a Petri dish. Both systems have the advantage to be relatively cheap. However, the benefits and drawbacks of each system are different.
A long time is required to start closed systems because (1) foraminifera need to be marked prior to the start of the experiment, (2) the water needs to be added in the 20-litres tanks and experimental bottles in advance so that it can reach the desired temperature, and (3) it is necessary to watch carefully for any leakage. The closed system has the advantage to be less time-consuming during the experiment, because only one day of manipulation per week (sampling of seawater and measurements of the physico-chemical parameters) is required, depending on the number of systems running at the same time.
The main advantage of the Petri dish system is that observations are possible during the experiment. However, the seawater needs to be renewed regularly (here, every 3 to 4 days), otherwise the conditions would change because of the very small volume of water (120 ml compared to 25 l in total for the closed system). Every time the water is changed, it is necessary to measure the conditions in the supernatant that we remove, but also in the water that we add. Therefore the time of manipulation is largely increased compared to the closed system (~ 2 days per week). The frequency of the seawater renewal depends on the desired stability and the time available for manipulation. Frequent changes of the water will help to keep stable conditions but will also be more time-consuming.

For the experiments in closed systems, it is essential for the foraminifera to be marked with a permanent label such as the calcein used in our experiments, otherwise newly calcified calcite can not be distinguished. For the Petri dish system, although we used marked specimens in our experiments, the labelling is not necessary because foraminifera are observed very easily, directly in the Petri dish with the stereomicroscope every time the water is changed. Adults can be removed as soon as they reproduced, or at least before the juveniles produced during the experiment have grown to a size where they can be confused with the adults added at the beginning of the experiment. The possible observation of the foraminifera during the experiment is also very important, because it gives the opportunity to stop an experiment in function of its evolution. For example, if no adults reproduce, it would save time to be able to decide in the course of the experiment to stop it. Also if we want only to obtain formation of new chambers in controlled conditions, it is possible to mark them with calcein before the start of the experiment; the observations during the experiment could lead to the decision to stop the experiment as soon as enough new chambers have been formed. This method saves time and, by reducing the experimental duration, the risks of undesired variations of the physico-chemical parameters are also reduced.

5.4. Recommendations

Depending on the aim of the study, preference should be given to the closed system or to the Petri dish system. The Petri dish system should be chosen preferably in the case of short-term experiments or if only calcification of new chambers in controlled conditions is required. For long-term experiments, e.g. when reproduction of deep-sea benthic foraminifera and growth of the juveniles to an adult size are expected, a closed system should be preferred. Within a system, the number of experimental bottles in serial succession is adaptable (from 1 bottle to 5 bottles per closed system for example). Numerous different experimental conditions such as faunal composition and food conditions can be tested simultaneously; monospecific experiments can be performed to avoid competition without multiplying the physico-chemical measurements, or different qualities and quantities of food can be added to different experimental bottles. In the case of Petri dishes, this would imply a multiplication of the number of Petri dishes and consequently a multiplication of the manipulation time needed to change the water and measure the physico-chemical parameters.

We think that the systems presented here are well adapted to measure $\delta^{18}O$ and trace metal in benthic foraminifera according to different conditions, such as temperature, salinity, trace

metal concentration in seawater, etc. For $\delta^{13}C$ calibration of foraminiferal shells, the maintenance of stable carbon isotopic composition in the seawater ($\delta^{13}C_{DIC}$) would be more challenging. Indeed, working with an "open" system implies that the natural annual cycle in the $\delta^{13}C$ of CO_2 in the atmosphere would be registered in the $\delta^{13}C_{DIC}$ of seawater. On the other hand, if a gas tight closed system is used, the food input will result in a decrease in $\delta^{13}C_{DIC}$ (and in pH) due to the degradation of the organic matter. A possible solution could be to perform the experiments in an "open" system but to shorten their duration so that the annual variations of atmospheric $\delta^{13}C$ remain negligible.

6. CONCLUSION AND IMPLICATIONS

In this paper, we presented two different systems to culture benthic foraminifera in controlled physico-chemical conditions: a closed system (CSI and CSII) and a Petri dish system (PD). Both systems are easy to set up and inexpensive so that proxy calibrations become easily accessible. The measurements of temperature, salinity, pH and alkalinity performed during 3 months experiments showed stable conditions for CSI and for part of the Petri dishes. For CSII, a decrease in pH was recorded during the experiments. We assumed that this decrease resulted from the fact that, CSII system was gas tight (not the case for CSI) and avoided equilibration of the CO_2, released by the degradation of the organic matter with the atmosphere.

These systems were set up to determine, in laboratory conditions, the influence of temperature on the oxygen isotopic composition of deep-sea foraminifera. *Bulimina marginata* reproduced in all the systems and the juveniles grew until a maximum size of 300 µm within the time of the experiments (3 months) so that measurement of the $\delta^{18}O$ of their shell was possible. The main difference between these two systems is 1) the time required for manipulation in the course of the experiment which is shorter for the CS system and 2) the possibility to look at the foraminifera only for the PD system.

Finally, these systems could be adapted to realise calibrations of other proxies. For example, they could used to perform experiments which aim to test the impact of salinity or different carbonate concentrations on the isotopic composition or trace metal incorporation of shallow water or deep-sea benthic foraminiferal shells.

CHAPITRE 5

CALIBRATION OF $\delta^{18}O$ OF DEEP-SEA BENTHIC FORAMINIFERAL SHELLS AS A TEMPERATURE PROXY: LABORATORY RESULTS

Barras C. [(1,2)*], Geslin E. [(1)], Michel E. [(2)], Jorissen F. [(1)], Duplessy J.-C. [(2)]

[(1)] Laboratory of Recent and Fossil Bio-Indicators (BIAF), UPRES EA 2644, Angers University, France, and Laboratory of Marine Bio-Indicators (LEBIM), Ile d'Yeu, France

[(2)] Laboratoire des Sciences du Climat et de l'Environnement (LSCE), CEA–CNRS-UVSQ, Gif-sur-Yvette, France

CHAPITRE 5

CALIBRATION OF $\delta^{18}O$ OF DEEP-SEA BENTHIC FORAMINIFERAL SHELLS AS A TEMPERATURE PROXY: LABORATORY RESULTS

1. INTRODUCTION

The potential utilisation of stable isotope ratios of carbonate fossils as a tool to reconstruct past changes in temperature was first suggested by Urey (1947) who studied the thermodynamic control of temperature on oxygen isotopic fractionation. Following this, several authors established paleotemperature equations on the basis of calibration with inorganically precipitated calcite (McCrea, 1950; O'Neil *et al*., 1969; Kim and O'Neil, 1997), mollusc shells (Epstein *et al*., 1953; Horibe and Oba, 1972) or foraminiferal shells (Emiliani, 1955; Shackleton, 1974; Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Bouvier-Soumagnac *et al*., 1986; Bemis *et al*., 1998). These first studies also showed that organisms induce a biological offset in the fractionation during calcification that was not observed for slowly precipitated inorganic calcite, which was in equilibrium with seawater composition. Introduced by Urey *et al*. (1951), this so-called vital effect appeared to be the result of metabolic and kinetic effects. Nevertheless, Duplessy *et al*. (1970) observed that this vital effect was constant for any given species. More recent studies pointed out that the microhabitat of the species (McCorkle *et al*., 1997) and the carbonate chemistry of the seawater (Spero *et al*., 1997; Zeebe, 1999; Rathmann and Kunhert, 2008) could also have an influence on the isotopic composition of foraminiferal shells. In natural environments, all the factors influencing the geochemistry of foraminiferal shells are interfering. Laboratory culture experiments can provide a powerful way to determine the impact of one single parameter while all the others remain constant. Numerous laboratory studies have been performed to study the oxygen isotopic fractionation in planktonic and shallow benthic foraminifera (e.g. Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1986; Chandler *et al*., 1996; Spero and Lea, 1996; Spero *et al*., 1997; Bemis *et al*., 1998). Until now, experiments with the deep-

sea benthic foraminiferal species used by paleoceanographers are very scarce (Wilson-Finelli *et al*., 1998; McCorkle *et al*., 2004).

In this chapter, we present oxygen isotopic measurements of deep-sea benthic foraminifera produced in culture experiments performed at different temperatures (between 4 and 19°C). *Bulimina marginata* was particularly successful in reproducing and precipitating calcite in our controlled conditions. We also sampled foraminifera of the same species from the field in different areas in a temperature range from 4 to 14°C. The aim of this study was to determine paleotemperature equations for specimens produced in experimental conditions and specimens grown in the field, in natural conditions, and to compare them to the theoretical paleotemperature equation for inorganic calcite established by O'Neil (1969) and modified by Shackleton (1974). We also hoped to obtain more insight into the ontogenetic effect on the isotopic composition of cultured, *in situ* and also fossil specimens by comparing different size fractions.

2. MATERIAL AND METHODS

2.1. Laboratory experiments

Laboratory experiments were performed in order to test the influence of different calcification temperatures on the $\delta^{18}O$ composition of foraminiferal shells. We applied two different strategies to obtain sufficient weight of calcite precipitated in controlled conditions (see Figure 4.1 in Chapter 4). Strategy 1 consisted in the introduction of adult specimens of *Bulimina marginata* (sensu lato) in the experiments with controlled conditions; after reproduction of these adults, the composition of juvenile shells produced and grown in controlled conditions could be analysed. For strategy 2, small labelled juveniles of *Bulimina marginata* (s.l.) were sampled from stored sediments and added to the experiments; next they calcified a large proportion of their shell under controlled conditions. Also in this second case, entire shells were analysed for $\delta^{18}O$ and $\delta^{13}C$, considering that the weight of the shell calcified previously to controlled conditions would be small.

Two different types of culturing systems were used to obtain reproduction and growth of deep-sea benthic foraminifera: a closed system and a Petri dish system (see Chapter 4 for details on the experimental setups). In total, three groups of experiments were performed, two in closed systems (CSI and CSII) and one in Petri dishes (PD), at different temperatures

(Table 5.1). Strategy 1 was utilized in all the experiments whereas strategy 2 was only used in PD-7.9, PD-10.2, PD-12.7 and PD-14.7.

Experiments	Temperature (°C)
CSI-7.9	7.9 ± 0.1
CSI-10.1	10.1 ± 0.1
CSI-12.7	12.7 ± 0.1
CSI-14.7	14.7 ± 0.1
CSII-4.1	4.1 ± 1.1
CSII-6.0	6.0 ± 0.5
CSII-9.3	9.3 ± 0.7
CSII-11.6	11.6 ± 0.3
CSII-17.2	17.2 ± 0.2
CSII-19.3	19.3 ± 0.1
PD-7.9	7.9 ± 0.1
PD-10.2	10.2 ± 0.1
PD-12.7	12.7 ± 0.2
PD-14.7	14.7 ± 0.1
PD-15.7	15.7 ± 0.1
PD-13.0	13.0 ± 0.1

Table 5.1: Summary of all the experiments performed under controlled conditions in the laboratory to study the influence of different calcification temperatures on the $\delta^{18}O$ composition of deep-sea benthic foraminifera. "CS" and "PD" correspond to closed systems and Petri dish systems, respectively.

The physico-chemical conditions (salinity, pH, alkalinity, $\delta^{18}O_{seawater}$) were controlled regularly during all the experiments. The variability of these different parameters is shown and discussed in Chapter 4, where Table 4.1 summaries the averages and variations of each parameter in each experiment. The main conclusions were that all the systems properly avoided significant evaporation which could increase the salinity and $\delta^{18}O_{seawater}$. The carbonate chemistry in all experiments from CSI and in PD-15.7 and PD-13.0 was very stable. The pH was in average around 7.9, which is lower than the usual pH of seawater. For CSII, a decrease of pH occurred in 6 experiments, resulting in a shift of 0.3 units between the start and the end of the experiments. For experiments PD-7.9 to PD-14.7, we observed a peak of pH and alkalinity at the start of the experiments. After this short event, conditions remained stable until the end of the experiments.

Juveniles of *Bulimina marginata* (s.l.) were produced under controlled conditions in all the experiments of CSI, CSII and PD (strategy 1) and growth of labelled juveniles was also obtained (strategy 2).

2.2. Field samples

In order to compare our experimental measurements with specimens of *Bulimina marginata* from the field, different area were investigated. The aim was to find stations were *Bulimina* was present and calcified at different temperature conditions. The cores used for this purpose were sampled in the Bay of Biscay (OB3G, SC1K, SC1S and OB9J), the Rhône prodelta (M2-10, M2-12 and M2-22), Cape Blanc (Sed-10, Sed-11 and Sed-15) and the Indian Ocean (MD76-128 and MD77-194) (Figure 5.1).

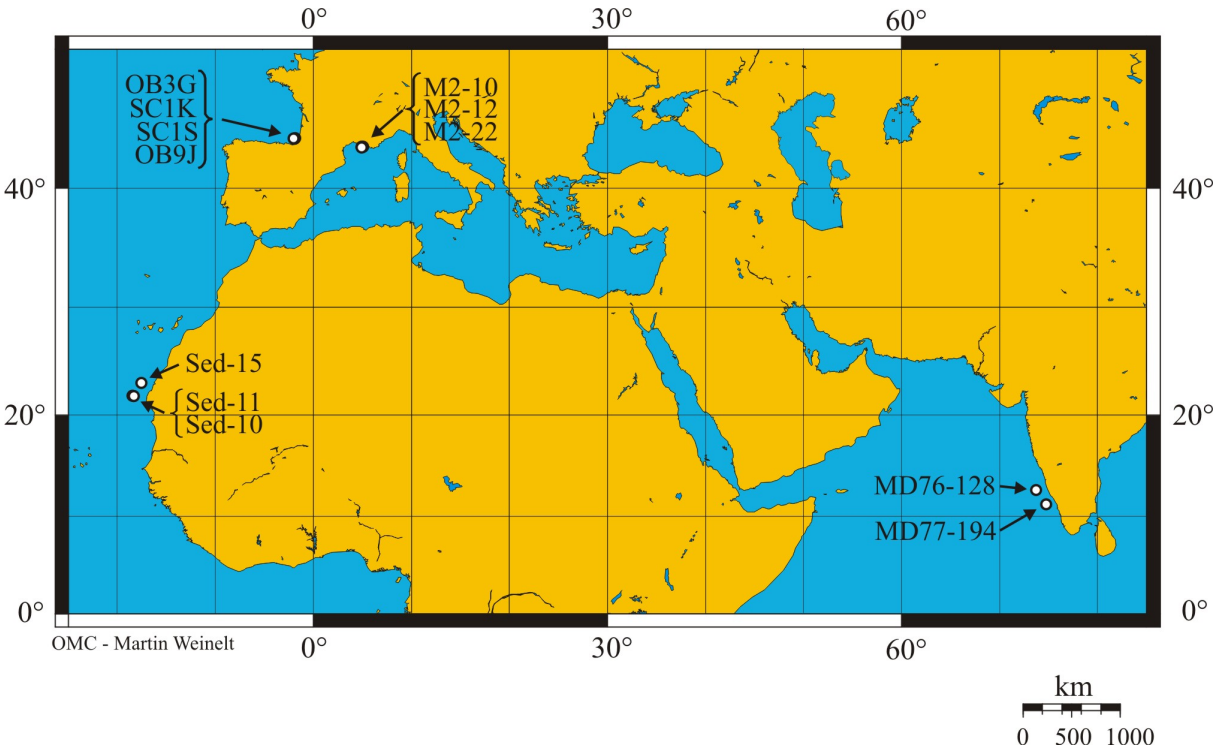


Figure 5.1: Geographical position of the cores used to sample B. marginata *at different temperatures for* $\delta^{18}O$ *analyses: Bay of Biscay (OB3G, SC1K, SC1S, OB9J), Rhône prodelta (M2-10, M2-12, M2-22), Cape Blanc (Sed-15, Sed-11, Sed-10) and Indian Ocean (MD76-128, MD77-194).*

2.2.1. Bay of Biscay

The Bay of Biscay (north-east Atlantic) is a semi-enclosed basin characterised by a vertical succession of rather homogeneous water masses (Ogawa and Tauzin, 1973). The stations sampled in this area in order to determine the isotopic composition of *B. marginata* are located in the axis of the Cap Breton canyon. Stations OB3G and SC1K, respectively at 445 and 650 m water depth, are situated in the Subarctic Intermediate Water (SAIW) which is present between 150 and 800 m water depth (Frew *et al*., 2000; van Aken, 2000). Station SC1S (790 m water depth) is positioned in transitional waters resulting from the mixing between the SAIW and the Mediterranean outflow water (MW) whereas station OB9J (860 m) is situated in the MW (Frew *et al*., 2000; van Aken, 2000). The Bay of Biscay is a mesotrophic basin (primary production around 145-170 g $C.m^{-2}.yr^{-1}$ according to Laborde *et al*., 1999) displaying two important bloom events each year. The major primary production event is the spring bloom that takes place at the end of winter or beginning of spring. The second, autumn phytoplankton bloom is much less well documented. During winter and summer, surface waters are generally rather oligotrophic. All the stations used for our study were sampled in June, between 1998 and 2001, to avoid the possible influence of different organic matter inputs throughout the year. The temperature at these stations ranges from 10.2 to 11.1°C (Table 5.2). The change of water masses with depth induces a difference of salinity between the shallowest station OB3G (35.6‰) and the deepest station OB9J (35.8‰) (Table 5.2).

At all stations, Rose Bengal stained specimens of *B. marginata* were sampled in the sediment intervals 0-0.25 or 0-0.5 cm except for OB9J where specimens were sampled between 2 and 10 cm depth.

2.2.2. Rhône prodelta

The Rhône prodelta is located in the Gulf of Lions, in the Northwestern Mediterranean Sea. This area is influenced by several physical parameters. The circulation of seawater and the sedimentation and particle distribution is controlled mainly by the wind regimes and also by the Liguro-Provençal current which flows south-westwards along the shelf break (Millot, 1990; Marsaleix, 1993). Northerly (Mistral) and north-westerly (Tramontane) winds, and S-SE winds (Marin) are predominant in the Gulf of Lions. Winds are responsible for the stratification of the surface layer that can persist from approximately April to October.

Stations 10, 12 and 22 were sampled in June 2005 during the Minercot cruise and the foraminiferal faunas at these stations were studied by Mojtahid *et al*. (submitted). These stations are located on the continental shelf at shallow water depth: 80, 58 and 74 m respectively for stations 10, 12 and 22. Therefore, the physico-chemical conditions are most probably less stable than at deep-sea areas. However, these three stations are located under the permanent thermocline in this area (Copin-Montégut and Bégovic, 2002) and they are outside of the direct influence of the plume of the Rhône River (B. Lansard, Pers. com.). Station 10 is located in the eastern part of the prodelta whereas stations 12 and 22 are in the western part. During the Minercot cruise (June 2005), *in situ* measurements recorded temperatures between 13.3 and 13.6°C at these stations and constant bottom water salinity at 38‰ (Mojtahid *et al*., submitted; Table 5.2). However, it is important to keep in mind that annual and seasonal variations of the temperature may lead to values between 13.0 and 15.0°C at 50 m water depth, and 13.0 and 13.5°C at 80 m depth in this area (Dyfamed site, Copin-Montégut and Bégovic, 2002). In the Rhône prodelta, the organic matter originates from different sources: continental sources, i.e. mainly the Rhône discharges, and marine biological production which is higher during spring (Gadel *et al*., 1990; Monaco *et al*., 1990; Buscail and Germain, 1997). Mojtahid *et al*. (submitted) performed measurements on the organic matter input and oxygenation conditions in the area. The bottom water in all the area is well oxygenated and the penetration depth varies between 2 and 6 mm sediment depth.
The Rose Bengal stained specimens of *B. marginata* used for isotopic measurements were sampled in the upper 5 centimetres of sediment.

2.2.3. Cape Blanc

Three stations (10, 11 and 15) of interest for our study were sampled in March 1994 during the Sedorqua cruise off Cape Blanc, North-West Africa. At stations 15, 11 and 10, respectively at 1000, 1200 and 1500 m water depth, the Mediterranean Outflow Water (MOW) is present (between 1000 and 1500 m depth north of 20°N) (Sarnthein *et al*., 1982). This area is characterised by an upwelling that is considered permanent throughout the year north of Cape Blanc (21°N) (Fütterer, 1983). Stations 10 and 11 are located around 21.5°N, in the main zone of organic matter deposition off Cape Blanc (Sarnthein *et al*., 1982; Fütterer, 1983; Lutze and Coulbourn, 1984) whereas station 15 is located further North at 25°N. Despite high organic carbon values in the clayey surface sediment at these stations, the bottom water oxygenation (between 147 and 191 $\mu mol.l^{-1}$) is elevated (Jorissen *et al*., 1998).

These stations exhibit a wider range of bottom water temperatures than the two previous areas with temperatures of 4.7, 5.8 and 6.5°C at stations 10, 11 and 15, respectively. Bottom water salinity is around 35‰ (Table 5.2).

The isotopic values recorded for *Bulimina marginata* at these three stations were measured by Griveaud (2007) at the LSCE in Gif-Sur-Yvette. For station 11, Rose Bengal stained specimens were analysed through all the sediment from the surface until 7 cm depth. The results showed no variability of the oxygen isotopic composition of the living foraminifera with sediment depth (Griveaud, 2007). For stations 15 and 10, *B. marginata* were sampled in the sediment interval 0-1 and 5-6 respectively.

Location	Core name	Latitude	Longitude	Water depth (m)	Sampling date	Temperature (T°C)	Salinity (‰)	δ18Ow (‰)	Specimens analysed	Size fractions (µm)
Bay of Biscay	OB3G	43°40'N	1°37'W	445	June 98	**11.1**	35.6	0.7	Rose Bengal stained	200-250 250-315 355-425
	SC1K	43°36'N	1°47'W	650	June 01	**10.5**	35.6	0.7	Rose Bengal stained	< 250 250-315 315-355 355-425
	SC1S	43°37'N	1°44'W	790	June 01	**10.3**	35.7	0.7	Rose Bengal stained	200-250 250-315 315-355 355-425
	OB9J	43°38'N	1°52'W	860	June 99	**10.2**	35.8	0.7	RB stained	no size
Rhône prodelta	M2-10	43°17'N	4°55'E	80	June 05	**13.5**	38	1.45	RB stained	250-315
	M2-12	43°16'N	4°42'E	58	June 05	**13.6**	38	1.45	RB stained	250-315
	M2-22	43°13'N	4°42'E	74	June 05	**13.3**	38	1.45	RB stained	250-315
Cape Blanc	Sed-15	23°43.6'N	17°15.6'W	1000	March 94	**6.5**	35.0	0.4	RB stained	no size
	Sed-11	21°28.9'N	18°57.2'W	1195	March 94	**5.8**	35.1	0.45	RB stained	no size
	Sed-10	21°25.0'N	18°04.2'W	1525	March 94	**4.7**	35.1	0.45	RB stained	no size
Indian Ocean	MD76-128	13°08'N	73°19'E	1712		**3.7**	34.85	0.05	Dead	150-250 250-315
	MD77-194	10°28'N	75°14'E	1222		**6.0**	34.94	0.1	Dead	150-250 250-315

Table 5.2: Summary of the field samples selected for $\delta^{18}O$ analyses of B. marginata*. Details on the location of the cores (geographical coordinates, water depth, sampling date), the physico-chemical conditions at the stations (temperature, salinity and $\delta^{18}O$ of seawater) and the type of individuals analysed (Rose Bengal/dead, size fractions) are indicated.*

2.2.4. Indian Ocean

The Arabian Sea, northwest of the Indian Ocean, is influenced by the important monsoonal system which leads to intense seasonal changes of surface water circulation and productivity. The particularly strong southwesterly winds of the summer monsoon result in extensive upwelling of nutrient-rich water in the western and northern regions of the Arabian Sea (Haake *et al*., 1993; Rixen *et al*., 1996; Rixen *et al*., 2000). During the northeast monsoon, the

surface circulation is reversed. Wind stress deepens the mixed layer which results in enhanced primary production, particularly in the northern and eastern regions (Smith *et al*., 1998; Qasim, 2000; Wiggert *et al*., 2000). The two stations sampled for specimens of *B. marginata*, MD76-128 and MD77-194, are located in the south east of the Arabian Sea (Figure 5.1). They are bathed by the North Indian Deep Water (NIDW) which spreads between the Oxygen Minimum Zone (OMZ) and the Antarctic Bottom Water (AABW), between 1200 and 3800 m (Kurbjeweit *et al*., 2000). The NIDW is saline, nutrient-rich and presents intermediate oxygen saturations in comparison to the OMZ and AABW. The organic matter input is high during northern hemisphere summer because of the winds producing upwelling off southwestern margin of India (Wyrtki, 1973; Divakar Naidu *et al*., 1999). Indeed, this upwelling is characterised by enriched nutrients in the surface waters and consequently higher productivity in the area. Temperatures at MD76-128 and MD77-194 stations are respectively 3.7 and 6.0°C and salinity is around 34.9‰ (Table 5.2).
The dead specimens of *B. marginata* selected for isotopic measurements were sampled in the 50 first of the top of each core which correspond to the Holocene.

2.3. Fossil samples

Thanks to C. Chiessi (Fachbereich Geowissenschaften, Bremen University), we had the opportunity to analyse fossils specimens of *B. marginata* in a core from the western South Atlantic Ocean. Core GeoB2107-3 was sampled off Brazil (27°18S, 46°46W) at 1050 m water depth. Specimens sampled from this core (142-143 cm sediment interval) allowed us to study the ontogenetic effect on the isotopic composition of the shell on a large range of sizes (from 200 to 900 µm). According to the age model based on ^{14}C measurements, the studied sediment interval corresponds to the period just after the Last Glacial Maximum (163-178 cm sediment depth) (C. Chiessi, Pers. com.).

2.4. Sample preparation and analyses

Oxygen and carbon isotopic analyses were conducted on entire specimens of *Bulimina marginata* (*marginata* and *aculeata* morphotypes measured separately). In order to study the ontogenetic effect on the $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios of the shells of deep-sea benthic foraminifera, specimens from culture, field and fossil samples were separated into different

size fractions before isotopic analyses. These size fractions were depending on foraminiferal sizes existing in the experiments or in the samples, and also on the quantity of foraminifera available of each size (a minimum weight of calcite of 20-40 µg was required for one measurement). Size fractions were not separated by sieving, but by measuring the length of the specimens with a microscale under the stereomicroscope. Table 5.3 presents the different size fractions used according to the source of foraminifera (i.e. culture, *in situ* or fossils). We also indicated the average size considered for each size fractions in order to represent graphically the ontogenetic effect on the oxygen isotopic composition of the shells.

	Size fractions	Average size (µm)
Culture experiments	< 100 µm	75
	100-150 µm	125
	< 150 µm	125
	= 150 µm	150
	> 150 µm	175
	150-200 µm	175
	200-250 µm	225
	> 250 µm	275
In situ	150-200 µm	175
	200-250 µm	225
	< 250 µm	225
	250-315 µm	282
	315-355 µm	335
	355-425 µm	390
Fossils	200-250 µm	225
	250-300 µm	275
	300-350 µm	325
	350-400 µm	375
	400-450 µm	425
	450-500 µm	475
	500-600 µm	550
	600-700 µm	650
	700-800 µm	750
	800-900 µm	850

Table 5.3: Summary of the different size fractions and average sizes established according to the source of the individuals of B. marginata *(culture experiments,* in situ *or fossils).*

For living specimens (from laboratory experiments), cleaning with methanol or ultrasonic bath was not necessary since the specimens analysed were born in experiments without sediment. It was also better to avoid any hard cleaning procedure because the foraminifera born in the culture were often rather fragile. Rose Bengal stained foraminifera were not cleaned with methanol because they appeared to be very clean (i.e. transparent, free of

mineral particles). On the other hand, fossil specimens were cleaned with methanol and ultrasonic baths. All the specimens from culture experiments, *in situ* and fossil samples were roasted at 350°C during 45 min to remove all the organic matter before analyses.

The $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios of foraminiferal calcite were measured with a mass-spectrometer at the LSCE (Gif-sur-Yvette) by F. Dewilde and F. Manssouri. Results are expressed as $\delta = ((R_{sample} - R_{standard}) / R_{standard}) * 1000$, where R is the isotopic ratio $^{18}O/^{16}O$ or $^{13}C/^{12}C$. The analytical precision of the $\delta^{18}O$ analyses is ±0.06‰ relative to the Vienna Pee Dee Belemnite (VPDB) standard (F. Dewilde, Pers. com.).

A minimum of 20 and 40 µg of carbonate shells were required to ensure the quality of the analyses with the Isoprim and VG-Optima mass-spectrometer, respectively. Depending on the size fraction considered, the number of specimens used for a measurement was very different. Considering cultured foraminifera, around 10 to 20 specimens were required for the largest size fraction (>250 µm) whereas around 150 specimens were used for the smallest size fractions (<150 µm).

The oxygen isotopic composition of the seawater was either directly measured in the case of the culture experiments (see Chapter 4) or estimated for the *in situ* samples. The $\delta^{18}O_w$ for the *in situ* samples from the Atlantic Ocean were estimated using close stations recorded in the GEOSECS database (Geochemical Ocean Sections Study). We used data from the literature to estimate the $\delta^{18}O_w$ at the stations sampled in the Mediterranean Sea (Duplessy and Fieux, 1972; Thunell *et al*., 1987) and the $\delta^{18}O_w$ in the Indian Ocean (Delaygue *et al*., 2001). The data were converted from VSMOW (Vienna Standard Mean Ocean Water) to VPDB by subtracting 0.27‰ (Hut, 1987).

In order to determine the relationship between temperature and the oxygen isotopic composition of specimens of *Bulimina*, we used a least squares regression. The oxygen isotopic composition was always expressed as the isotopic difference between foraminiferal shell and seawater ($\delta^{18}O_f$-$\delta^{18}O_w$), where both values are expressed relative to VPDB. We applied linear regression to our data sets since it provided equally good fits as quadratic regression. The choice of linear or quadratic equations was discussed by Bemis *et al*. (1998). The coefficient of determination (R^2) and the standard error on the slope and intercept were calculated and are indicated for each equation. The equations are compared with the paleotemperature equation obtained by O'Neil *et al*. (1969) for inorganic calcite and adapted

by Shackleton (1974) as the following paleotemperature equation for a temperature range between 0 and 30°C:

$$T = 16.9 - 4.38\ (\delta^{18}O_f - \delta^{18}O_w) + 0.10\ (\delta^{18}O_f - \delta^{18}O_w)^2$$

To compare this equation with the one obtained in our study, we applied least squares regression for the range of temperatures considered.

We also investigated the influence of shell size and the oxygen ($\delta^{18}O_f$-$\delta^{18}O_w$) and carbon ($\delta^{13}C$) isotopic composition of foraminiferal shell. The fossil specimens were only analysed in order to study the ontogenetic influence, and the paleo-$\delta^{18}O_w$ was not estimated.

3. RESULTS

3.1. Intrageneric variability: B. marginata *f.* marginata *vs.* B. marginata *f.* aculeata

In the laboratory experiments, adult specimens of *B. marginata* forma *marginata* and *B. marginata* forma *aculeata* were introduced to complete strategy 1. Depending on the experiments, juveniles of either *B. marginata* f. *marginata* and/or *B. marginata* f. *aculeata* were produced. However, measurements of the isotopic composition of *B. marginata* f. *aculeata* were scarcer because less specimens of this morphotype were produced. In order to evaluate the similarity or difference in the $^{18}O/^{16}O$ ratios of these two morphospecies, we compared the $\delta^{18}O$ values of specimens of *B. marginata* f. *marginata* and *B. marginata* f. *aculeata* that calcified in exactly the same conditions in our experiments (same experiment and same temperature). Unfortunately, when juveniles of a*culeata* morphotype were produced, the quantity of specimens was not sufficient to be able to divide them into different size fractions. Figure 5.2 presents the oxygen isotopic composition of the juveniles of *marginata* morphotype from all size fractions and *aculeata* morphotype (all sizes) for the experiments where specimens of both morphotypes were produced; these conditions were encountered in CSI-12.7, CSI-14.7, CSII-17.2 and CSII-19.3.

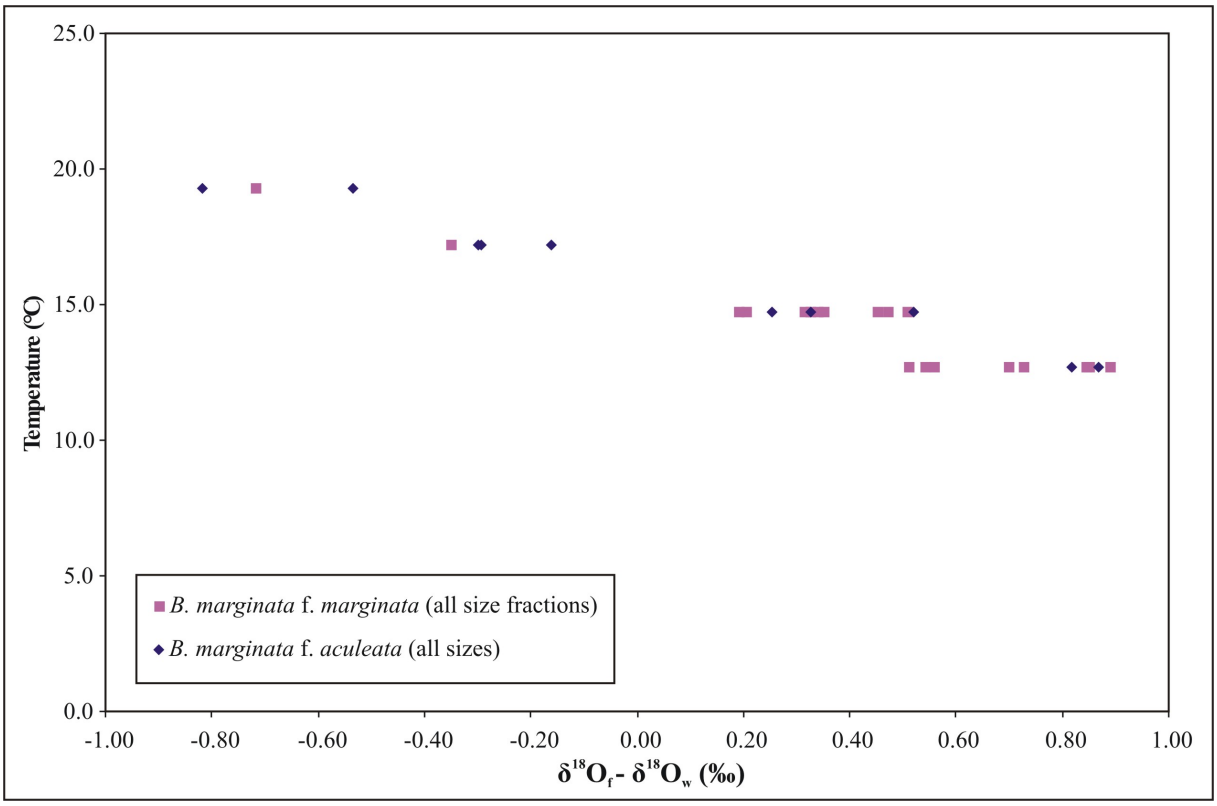


Figure 5.2: Experimental $\delta^{18}O_f$-$\delta^{18}O_w$ values versus temperature for cultured specimens of B. marginata *f.* marginata *and* B. marginata *f.* aculeata *that calcified their entire test in the same experiments (CSI-12.7, CSI-14.7, CSII-17.2 and CSII-19.3). All sizes are plotted together.*

The $\delta^{18}O$ of specimens of *aculeata* morphotype appears to be slightly heavier than the $\delta^{18}O$ of specimens of *marginata* morphotype. However, this small difference may come from the comparison of different size fractions. Anyway, the number of measurements of *B. marginata* f. *aculeata* specimens is not sufficient to insure that there are no intrageneric differences in the fractionation. Woodruff *et al*. (1980) and Berger and Wefer (1991) mentioned the possibility of species-specific isotopic effects in one given genus (e.g. for *Uvigerina* or *Pyrgo*). Significant $\delta^{18}O$ differences of more than 0.20‰ were measured between *U. mediterranea* and *U. peregrina* (Schmiedl *et al*., 2004; Fontanier *et al*., 2006). This effect would increase the scatter of the $\delta^{18}O$ values obtained, introducing a possible bias in the establishment of the relation between temperature and $\delta^{18}O$ of foraminiferal shell. Therefore, we decided in the rest of this chapter to process only the data of *B. marginata* f. *marginata* (referred as *B. marginata*) for which more data were available, and not to present data for *B. marginata* f. *aculeata*.

3.2. $\delta^{18}O$ composition of **B. marginata** *in the different culture experiments (strategy 1)*

3.2.1. Comparison between the three systems

In order to compare the oxygen isotopic composition of foraminifera that calcified the totality of their test in the 3 different systems (CSI, CSII and PD) in controlled experimental conditions, we decided to group together all the smallest size fractions measured (<100, 100-150, =150 µm, see Table 5.3) into one size fraction called "≤150 µm size fraction", and the size fractions 150-200 and >150 µm together. The size fraction >250 µm could not be used for this comparison between the different systems since foraminifera of this size were only obtained for the system CSI. Therefore, only 3 different size fractions are considered: ≤150, 150-200 and 200-250 µm.

For each of the 3 size fractions, we plotted the oxygen isotopic composition of the foraminiferal shell of *B. marginata* ($\delta^{18}O_f$-$\delta^{18}O_w$) in function of the different temperature tested in the experiments (Figure 5.3 a-b-c). The $^{18}O/^{16}O$ composition of *B. marginata* appears similar in the 3 systems for a given size fraction and a given temperature (Figure 5.3, Appendix 5.1). When sufficient data were available, we established the relationship between the calcification temperature and $\delta^{18}O$ composition for a given system and a given size fraction. This was only possible for CSI for the size fraction 200-250 µm (not enough data available for PD and CSII). We used Lin's test (Lin, 1989) to estimate the concordance of the regression lines between the systems PD, CSI and CSII for size fractions ≤150 and 150-200 µm. We obtained concordance correlation coefficients always higher than 0.990 which confirms the similarity of the data obtained with the 3 systems for these 2 size fractions. From the statistical tests and the data presented in Figure 5.3 a-b-c, we can conclude that the $\delta^{18}O$ composition of *B. marginata* specimens from a same size fraction that calcified in a given temperature is identical in the three systems.

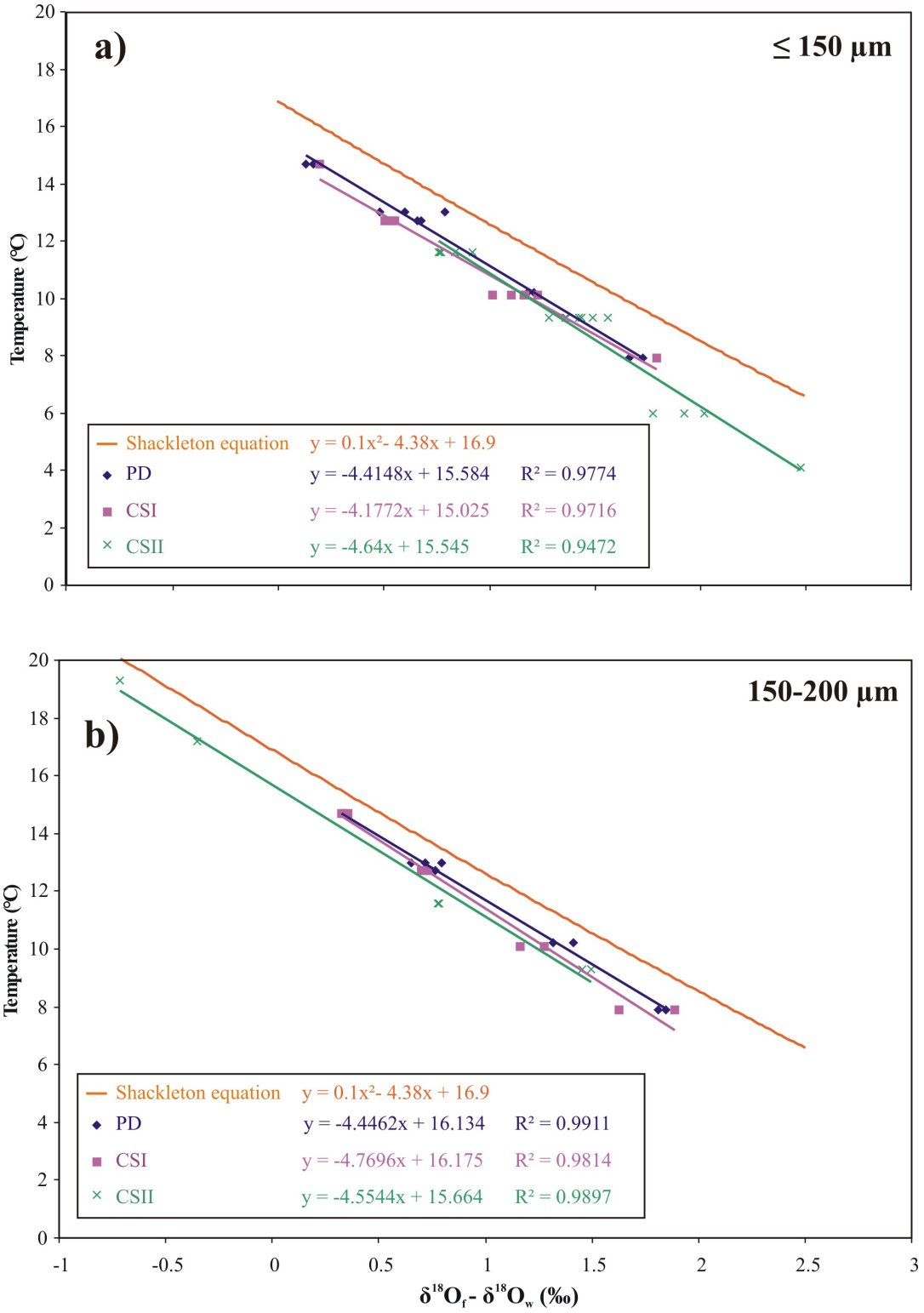


Figure 5.3: Comparison between the experimental data obtained ($\delta^{18}O_f$-$\delta^{18}O_w$ versus temperature) with the three different systems (PD, CSI and CSII) according to three different size fractions: (a) ≤ 150 µm, (b) 150-200 µm, and (c) 200-250 µm. The Shackleton equation for equilibrium calcite is indicated.

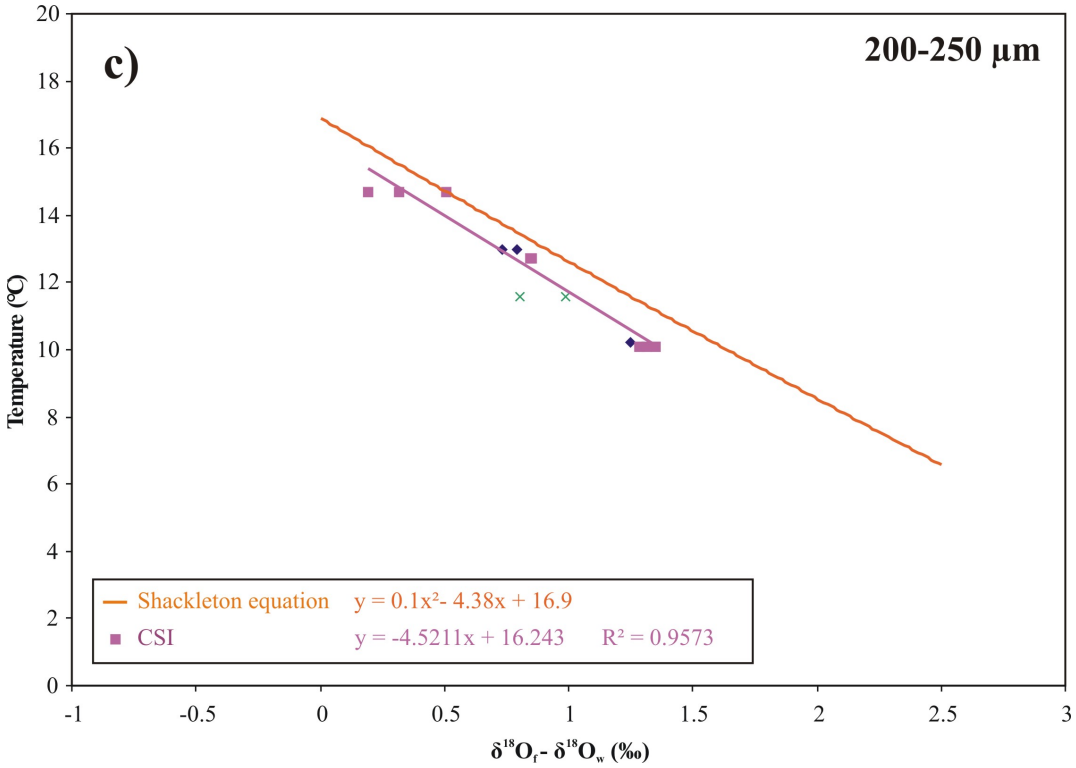


Figure 5.3 (Continued)

3.2.2. Influence of the temperature on the $\delta^{18}O$ composition of cultured foraminifera

Because the results obtained for the 3 systems are comparable, we can combine them to determine the influence of the calcification temperature on the isotopic composition of *B. marginata* in experimental conditions, for different size fractions (Figure 5.4 a-b-c-d).

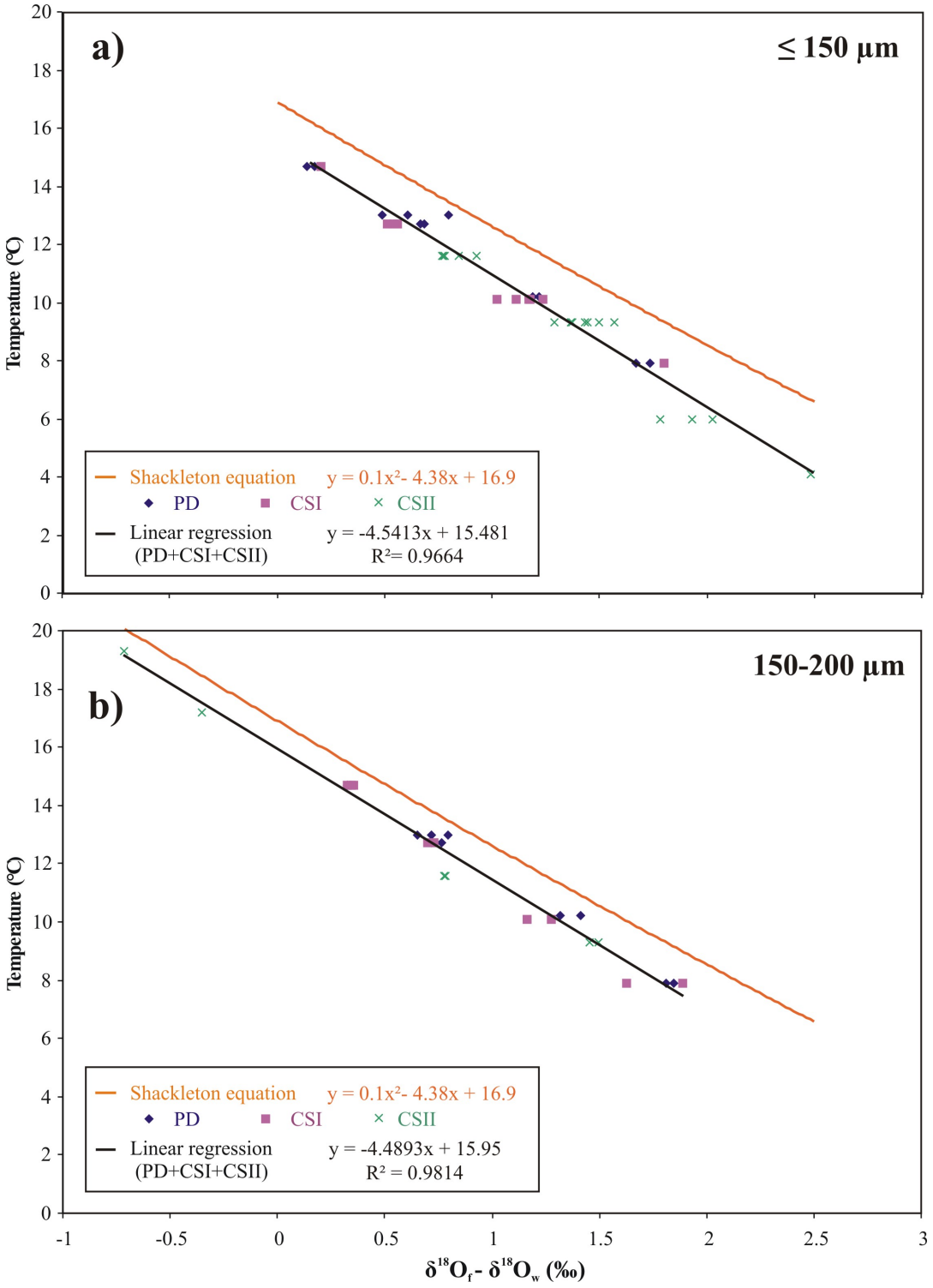


Figure 5.4: Calibration equations of $\delta^{18}O_f$-$\delta^{18}O_w$ versus temperature of cultured specimens of B. marginata *from the three systems together according to four different size fractions: (a) ≤ 150 µm, (b) 150-200 µm, (c) 200-250 µm, and (d) > 250 µm. The Shackleton equation for equilibrium calcite is indicated.*

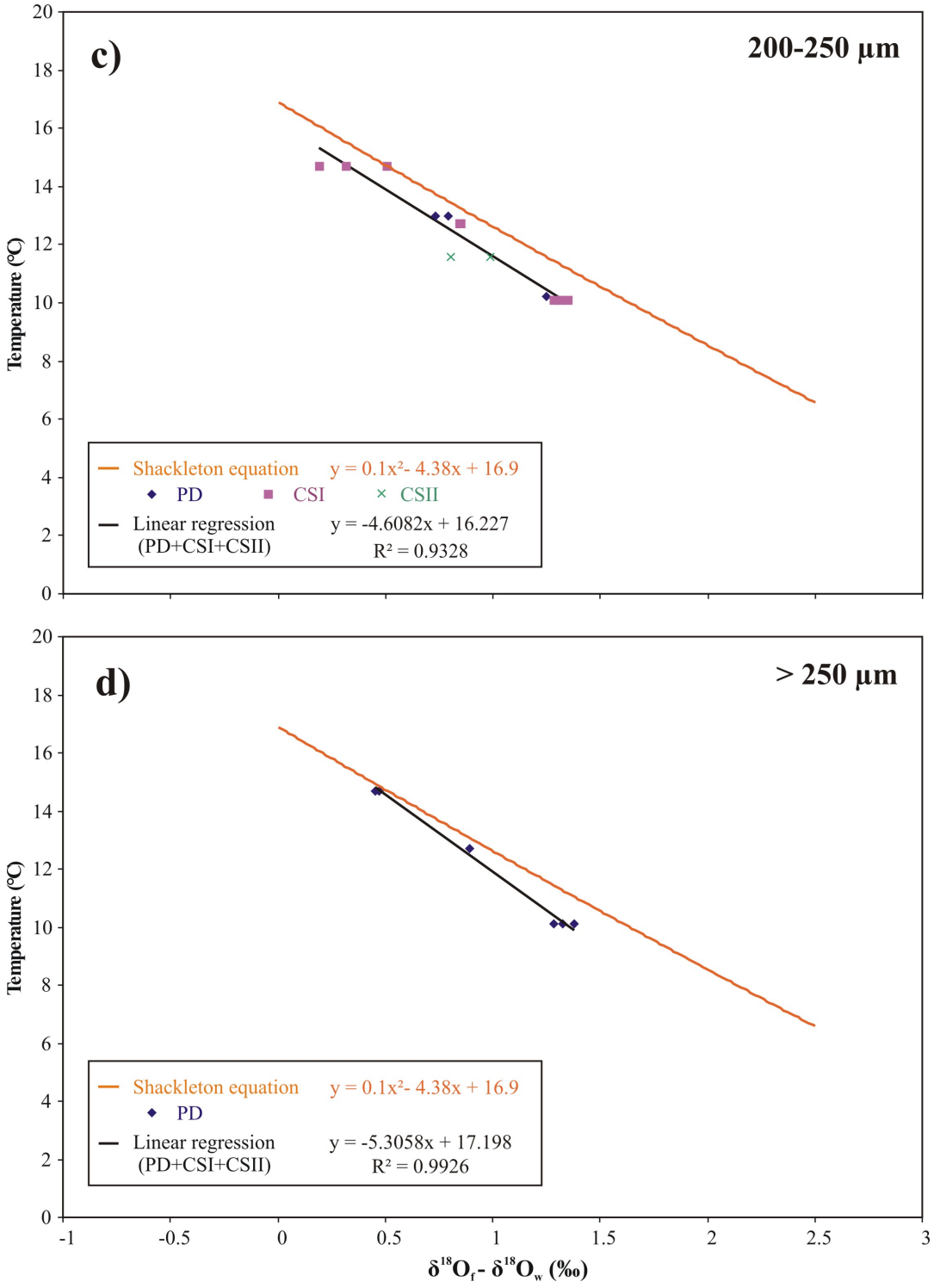


Figure 5.4 (Continued)

In Figure 5.5, we present the values of the slopes and intercepts (and their uncertainties) of the different calibration equations ($\delta^{18}O_f$-$\delta^{18}O_w$ vs. temperature) determined from our culture and *in situ* data. In order to compare our linear regression curves with the quadratic Shackleton

equation, we approximated the quadratic equation with linear curve (least squares regression). The exact equation of the linear fit depends on the temperature ranges considered to determine our experimental and field calibration equations (e.g. different according to the size fraction considered). This explains why the slope and intercept of the linear equation of the Shackleton equation are not constant in Figure 5.5.

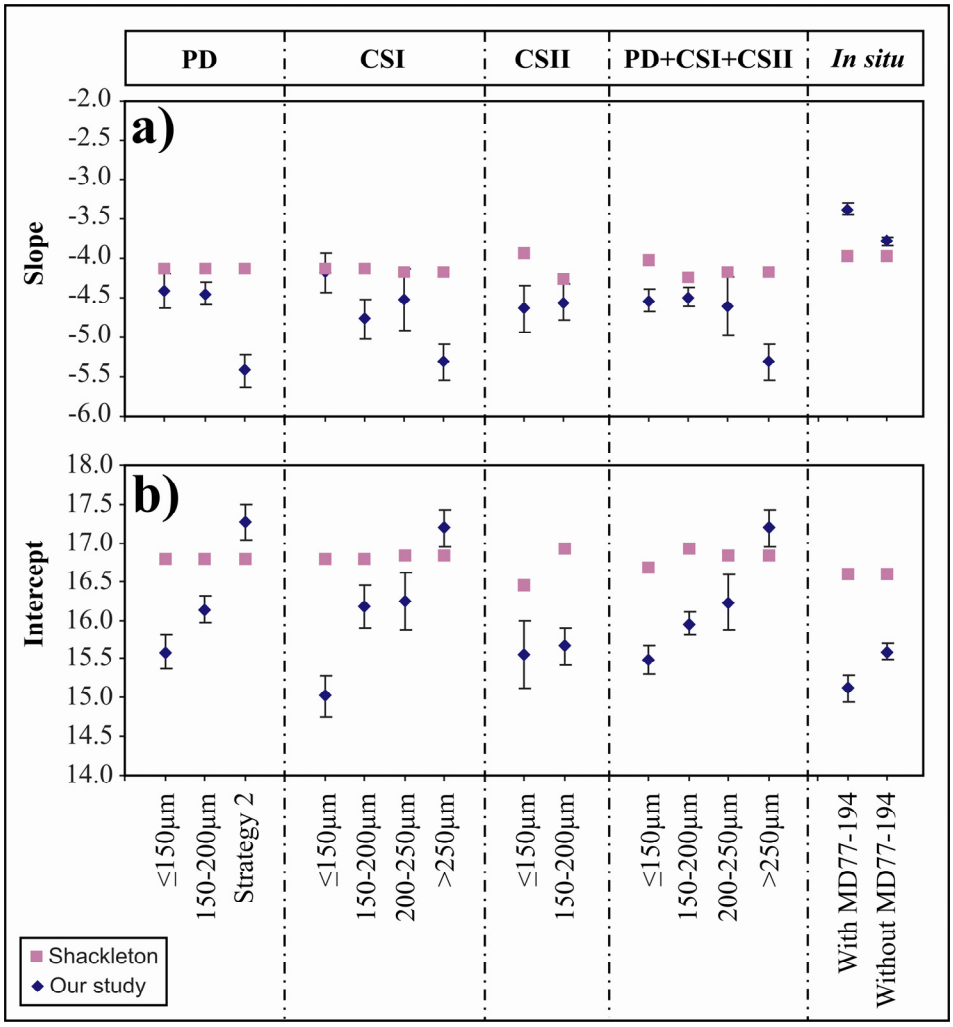


Figure 5.5: Summary of the slopes (a) and intercepts (b) of all the equations for each system separately, for the three systems combined and for the in situ *samples. These results are compared to the slope and intercept of linear regression of the Shackleton equation for the experimental temperature range (see the text for more details).*

The linear equations which best describe the relationship between temperature and isotopic composition of cultured foraminiferal shells (PD, CSI and CSII data combined) exhibit similar slopes, ranging from 4.49 to 4.61 (Figures 5.4 and 5.5 a), for size fractions ≤150 µm, 150-200 µm and 200-250 µm considering the standard errors on the slope estimates. For the

size fraction >250 µm, we can notice a slight increase in the slope of the regression equation (5.31±0.23). There is a significant increase in the intercept values with increasing size fractions (15.48, 15.95, 16.23 and 17.20 respectively for the size fractions ≤150, 150-200, 200-250 and >250 µm; Figures 5.4 and 5.5 b). We can deduce that the $^{18}O/^{16}O$ composition of the shell depends on the size of the specimens due to biological effects that are independent of the temperature of the experiment.

If the linear regressions obtained on the basis of our experimental data are compared with calcite in equilibrium (Shackleton, 1974), we can see that they are all nearly parallel except for the >250 µm size fraction. However, the linear regression of this size fraction is badly defined because we dispose of only few samples. The values of the slopes of the experimental equations are close to the slope of the regression lines applied to the quadratic relationship of Shackleton (1974), for the range of temperature of the experimental data. We also observe that the larger the size fraction is, the closer the regression lines are to the paleotemperature equation (Figure 5.5 b).

3.3. $\delta^{18}O$ composition of labelled **B. marginata** *in the different culture experiments (strategy 2)*

Strategy 2 consisted in the inoculation of small labelled juveniles of *B. marginata* in PD-7.9, PD-10.2, PD-12.7 and PD-14.7 respectively at 7.9, 10.2, 12.7 and 14.7°C. A large part of these juveniles grew new chambers and they reached on average 200 µm in length. Estimations based on the calculation of the shell area suggest that around 80% of the totality of the shell was calcified in the controlled conditions (see Chapter 4). Figure 5.6 presents the isotopic composition of the entire shells of the labelled grown juveniles according to their calcification temperatures. If we look at the regression line, we notice that it is not parallel to the Shackleton equation, contrary to the specimens that calcified their entire test in physico-chemically controlled experiments (strategy 1). In the case of strategy 2, the part of the shell (~ 20%) that was formed previously to the start of the experiments, at a different temperature, has obviously an influence on the isotopic composition of entire shells. Apparently, the contribution of the labelled part of the shell (calcified in uncontrolled conditions) to the whole shell isotopic value is not negligible and must be taken into account (see § 4.2).

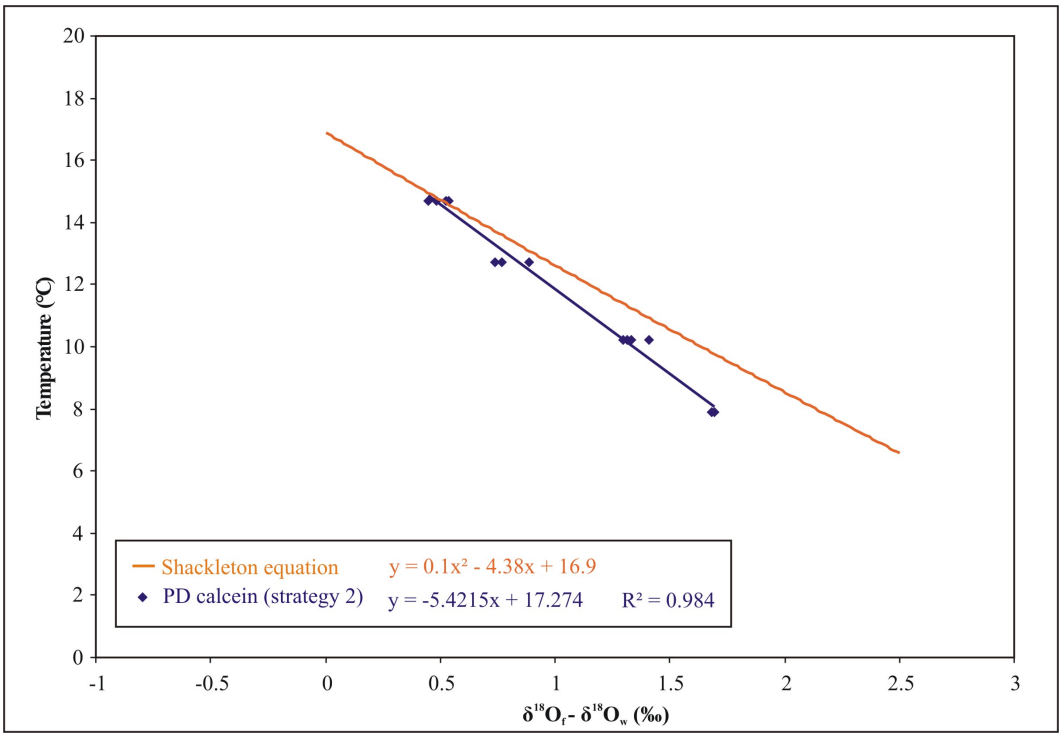


Figure 5.6: Experimental $\delta^{18}O_f$-$\delta^{18}O_w$ values versus temperature for labelled specimens of B. marginata *that calcified ~80% of their shell in controlled conditions (strategy 2). The Shackleton equation for equilibrium calcite is indicated.*

3.4. $\delta^{18}O$ composition of B. marginata *in the field*

If we have a look at the $\delta^{18}O$ measurements obtained from *in situ* samples, we can see that we succeeded to analyse samples from different *in situ* temperatures (Appendix 5.1 and Figure 5.7). The range of temperature from *in situ* samples is smaller than the temperature tested in the laboratory (from 3.7 to 13.6°C for *in situ* samples and from 4.1 to 19.3°C for culture samples).

Specimens of *B. marginata* sampled in cores from the Bay of Biscay (except for stations OB9J and OB3D), the Rhône prodelta and the Indian Ocean presented sizes between 150 and 425 µm. Foraminiferal size from the three stations of Cape Blanc (Sed-10, 11 and 15) and two stations of the Bay of Biscay (OB9J and OB3D) was not known. Specimens from the Bay of Biscay (stations OB3G, SC1K and SC1S) were measured, counted and weighted before each isotopic measurement (Table 5.4). We could then calculate the average weight of one Rose Bengal stained specimen of *B. marginata* according to the different size fractions, e.g. 4.2 and 6.4 µg/specimen for the 315-355 and 355-425 µm size fractions, respectively (Table 5.4). These data were used to obtain a rough idea of the foraminiferal size in the samples where

Rose Bengal specimens were not measured before being analysed. From the weight of the samples before the isotopic measurement and the number of foraminifera per sample, we were able to calculate the approximate weight per specimen for the samples of one station of Cape Blanc (Sed-11, data were not available for the other two stations) and stations OB9J and OB3D (Table 5.4), and consequently estimate the average size of the specimens of these samples. For the samples from Cape Blanc (Sed-11), the average weight of one specimen was estimated around 13.3 µg which means that specimens used for isotopic measurements were larger than 400 µm. For stations OB9J and OB3D, the estimated sizes are respectively around 350 µm (~ 5.2 µg/sp) and between 200 and 250 µm (~ 2.3 µg/sp) (Table 5.4).

Size fraction	Station	Nb of specimens per sample	Weight per sample (µg)	Estimated weight per specimen (µg)
200-250 µm	OB3G	22	46	
	OB3G	21	44	
	SC1S	17	44	
	Average	**20.0**	**44.7**	**2.2**
250-315 µm	OB3G	15	48	
	OB3G	18	47	
	OB3G	18	48	
	SC1K	14	48	
	SC1K	19	69	
	SC1S	18	73	
	SC1S	19	60	
	Average	**17.3**	**56.1**	**3.2**
315-355 µm	OB3G	13	45	
	OB3G	13	55	
	OB3G	13	47	
	SC1K	10	41	
	SC1K	11	52	
	SC1S	12	56	
	SC1S	13	61	
	Average	**12.1**	**51.0**	**4.2**
355-415 µm	OB3G	7	45	
	OB3G	8	50	
	OB3G	8	52	
	SC1K	10	70	
	SC1S	9	52	
	Average	**8.4**	**53.8**	**6.4**
Unknown	OB9J	13	67	**5.2**
Unknown	OB3D	7	16	**2.3**
Unknown	Sed-11	3	59	
	Sed-11	5	42	
	Sed-11	4	71	
	Sed-11	4	23	
	Sed-11	2	42	
	Sed-11	3	66	
	Sed-11	4	69	
	Sed-11	4	36	
	Sed-11	3	49	
	Sed-11	5	53	
	Sed-11	4	34	
	Sed-11	4	29	
	Sed-11	2	50	
	Average	**3.6**	**47.9**	**13.3**

Table 5.4: Estimates of the weight per specimen (µg), according to the weight of the samples used for isotopic measurements and the number of specimens per sample, for stations from the Bay of Biscay and Cape Blanc.

To compare the data of *in situ* samples with the data of the culture experiments taking into account size fractions (size between 150 and 250 µm), only a very small number of data is available for the *in situ* conditions (Figure 5.7 a, empty symbols). However, the *in situ* results from this size fraction fit well with the Shackleton equilibrium equation. Therefore, foraminifera from this size fraction sampled in the field appear to be big enough to correspond to the paleotemperature equation (relatively small biological effect due to ontogeny).

Considering both size fractions 150-250 and 250-315 µm, we can see that the scatter of the data obtained at 6.0°C (core MD77-194) is increasing (Figure 5.7 a). If we consider now all the size fractions together (150 to 425 µm + samples where foraminifera were not measured), we obtain a regression line between the temperature and the $\delta^{18}O_f$-$\delta^{18}O_w$ with a slope that is lower than for the Shackleton equation and than in our experiments (Figure 5.7 b and 5.5 a). The lower slope value is partly triggered by the Rhône data, which are the "high temperature" samples that we measured to establish the field calibration. The stations sampled in this area are located at a relatively shallow water depth (50-80 m) compared to the other field samples (450-1700 m). The conditions of temperature and salinity at the Rhône stations are certainly varying according to seasons and to the Rhône outflow. Therefore, the Rhône data should be considered with care since the temperature may have slightly changed during the calcification of the specimens analysed here. This problem is not encountered in the deep sea stations since the temperature and salinity conditions should be more stable.

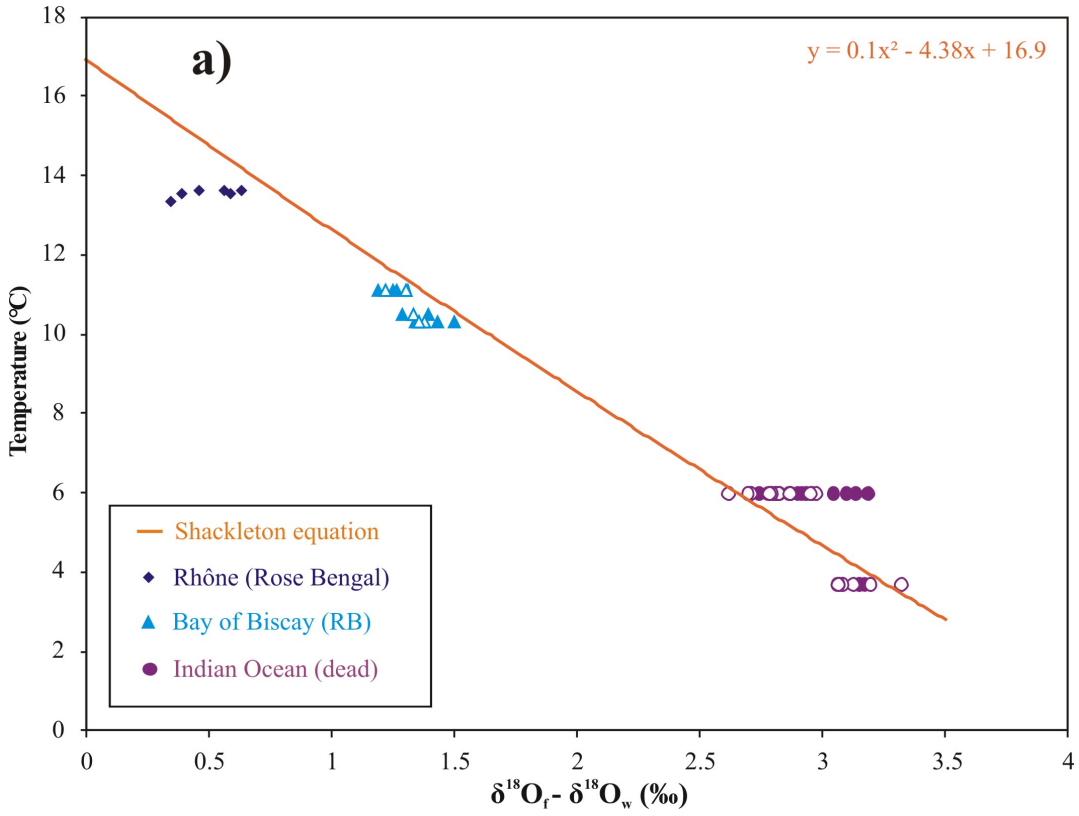


Figure 5.7: $\delta^{18}O_f$-$\delta^{18}O_w$ data versus temperature for specimens of B. marginata *sampled in the field: (a) only the specimens from size fractions 150-250 µm (empty symbols) and 250-315 µm (full symbols), (b) all data from all the locations (all sizes), and (c) all data from all locations except core MD77-194 from the Indian Ocean.*

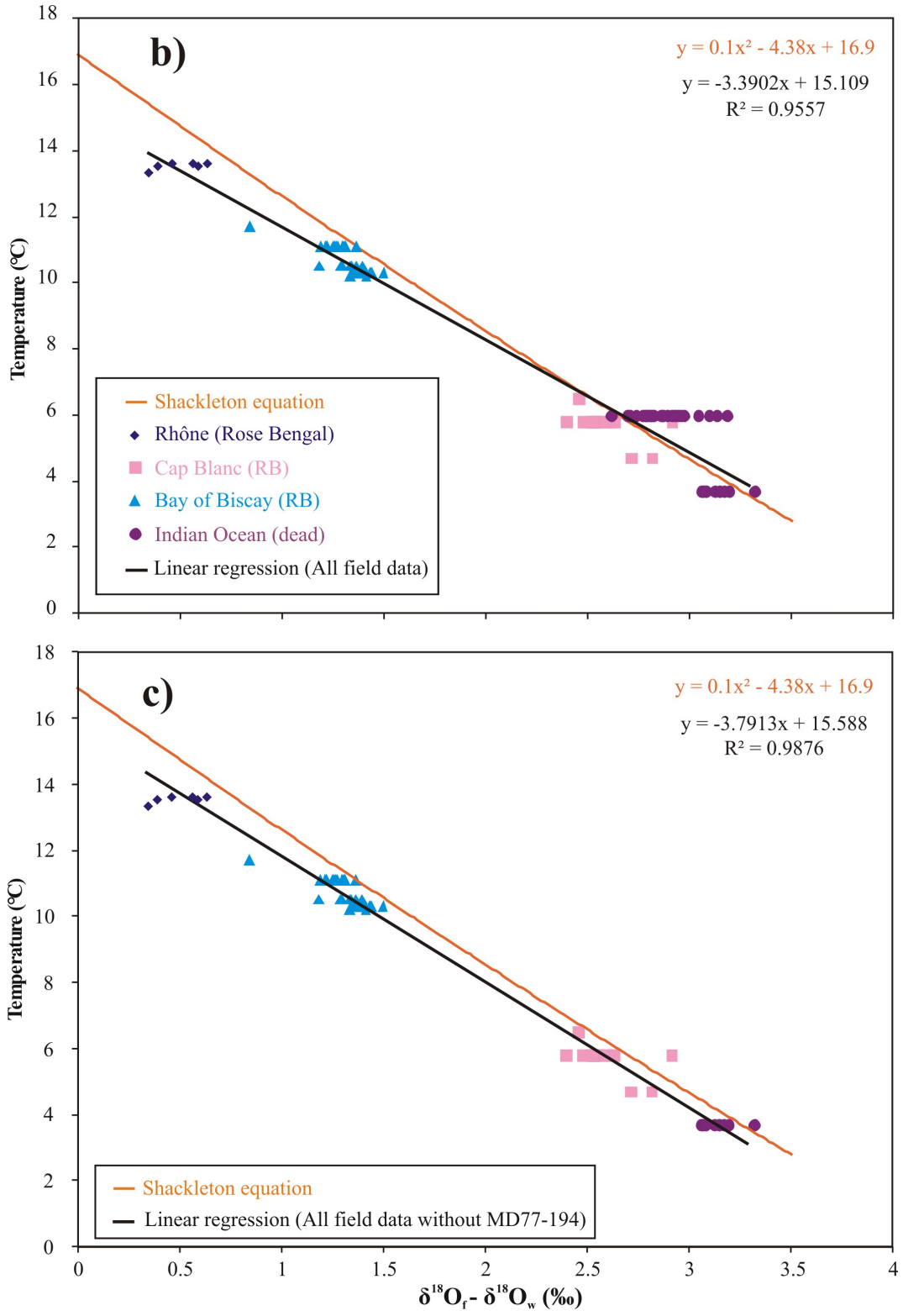


Figure 5.7 (Continued)

If we look into more details at the data obtained for MD77-194, we observe that there is large scatter in the $\delta^{18}O$ composition of *B. marginata*. Specimens were sampled in the 50 first centimetres of the core, every 5 cm. Figure 5.8 a presents all the $^{18}O/^{16}O$ ratios obtained in core MD77-194 through the 50 first centimetres and shows that the scatter observed is not especially due to the size fraction considered but also to noise in the general signal (variations up to 0.4‰ at a given depth and given size fraction). The same was observed for the signal obtained with *Uvigerina* with a scatter in the data during the Holocene. Specimens from core MD76-128 were analysed with the same mass spectrometer as specimens from core MD77-194 but the measurements are much less scattered (Figure 5.8 b). This tends to prove that the noise recorded in the signals from MD77-194 is coming from the core and not from the isotopic measurements. If we consider now all the *in situ* samples except the data from core MD77-194 (Figure 5.7 c), we obtain a correlation between the isotopic composition of *B. marginata* and the temperature of calcification which is very close to the relation of Shackleton.

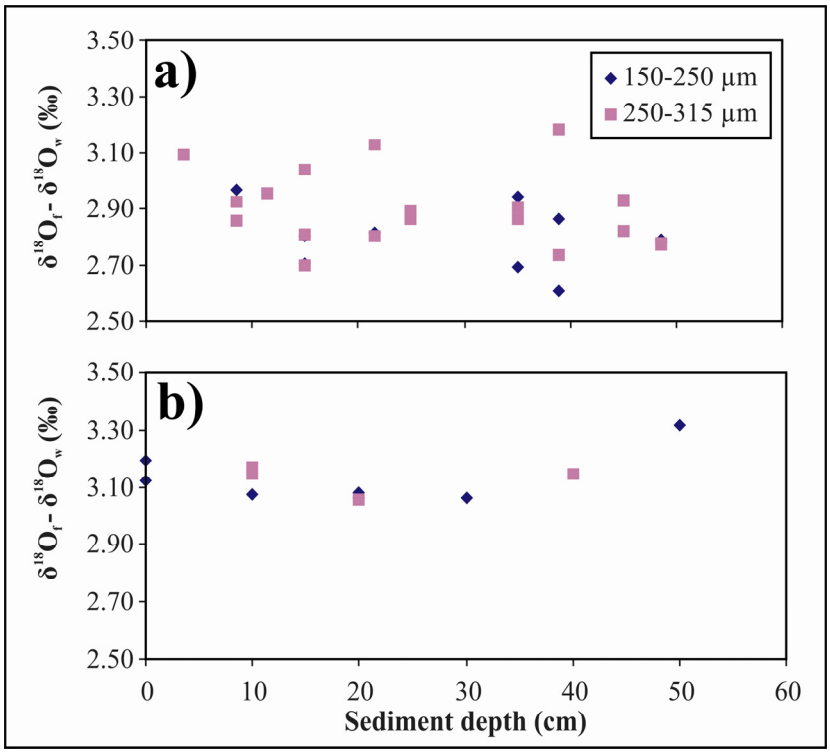


Figure 5.8: Composition of the specimens of B. marginata *($\delta^{18}O_f$-$\delta^{18}O_w$) analysed versus sediment depth within the 50 first centimetres top cores of (a) MD77-194 and (b) MD76-128. Note that the scatter observed for MD77-194 is much wider than for MD76-128 and shows no trend with sediment depth or according to the size fraction considered.*

3.5. *Effect of the size on the isotopic composition of cultured,* in situ *and fossil specimens of* B. marginata

3.5.1. $\delta^{18}O$ versus size

To have a better insight to the effect of the size, we plotted the $\delta^{18}O_f$-$\delta^{18}O_w$ for *B. marginata* in function of the average size of the different size fractions (see Table 5.3). We grouped the data obtained for CSI and PD since the physico-chemical conditions (temperature, salinity, pH and alkalinity were identical and we demonstrated that there was no significant difference in the composition of foraminifera according to the 3 systems (Figure 5.9 a). Data for the specimens calcified in CSII are presented separately (Figure 5.9 b) because the physico-chemical conditions in these experiments were slightly different from CSI and PD (pH decrease during the experiments). Figure 5.9 c-d present the $\delta^{18}O_f$-$\delta^{18}O_w$ versus average size for specimens from the Bay of Biscay and fossil specimens, respectively.

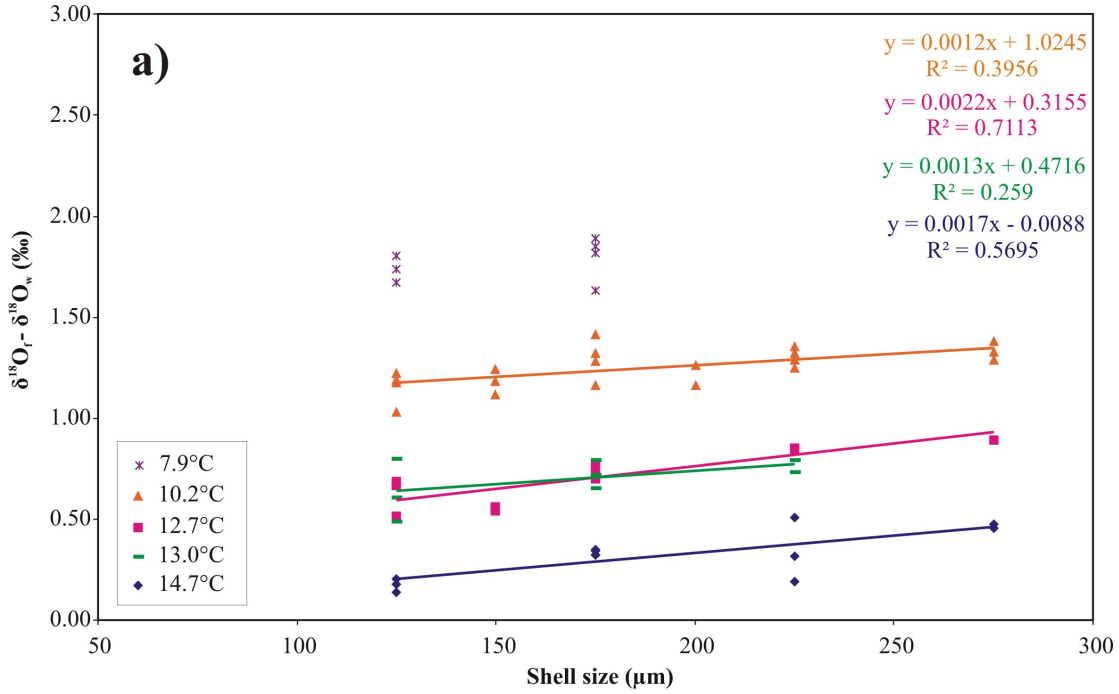


Figure 5.9: Shell size effect on the oxygen isotopic composition ($\delta^{18}O_f$-$\delta^{18}O_w$ or $\delta^{18}O$) of B. marginata *from (a) PD and CSI experiments together, (b) CSII experiments, (c) field samples, and (d) fossil samples. Note the different scales in the four figures.*

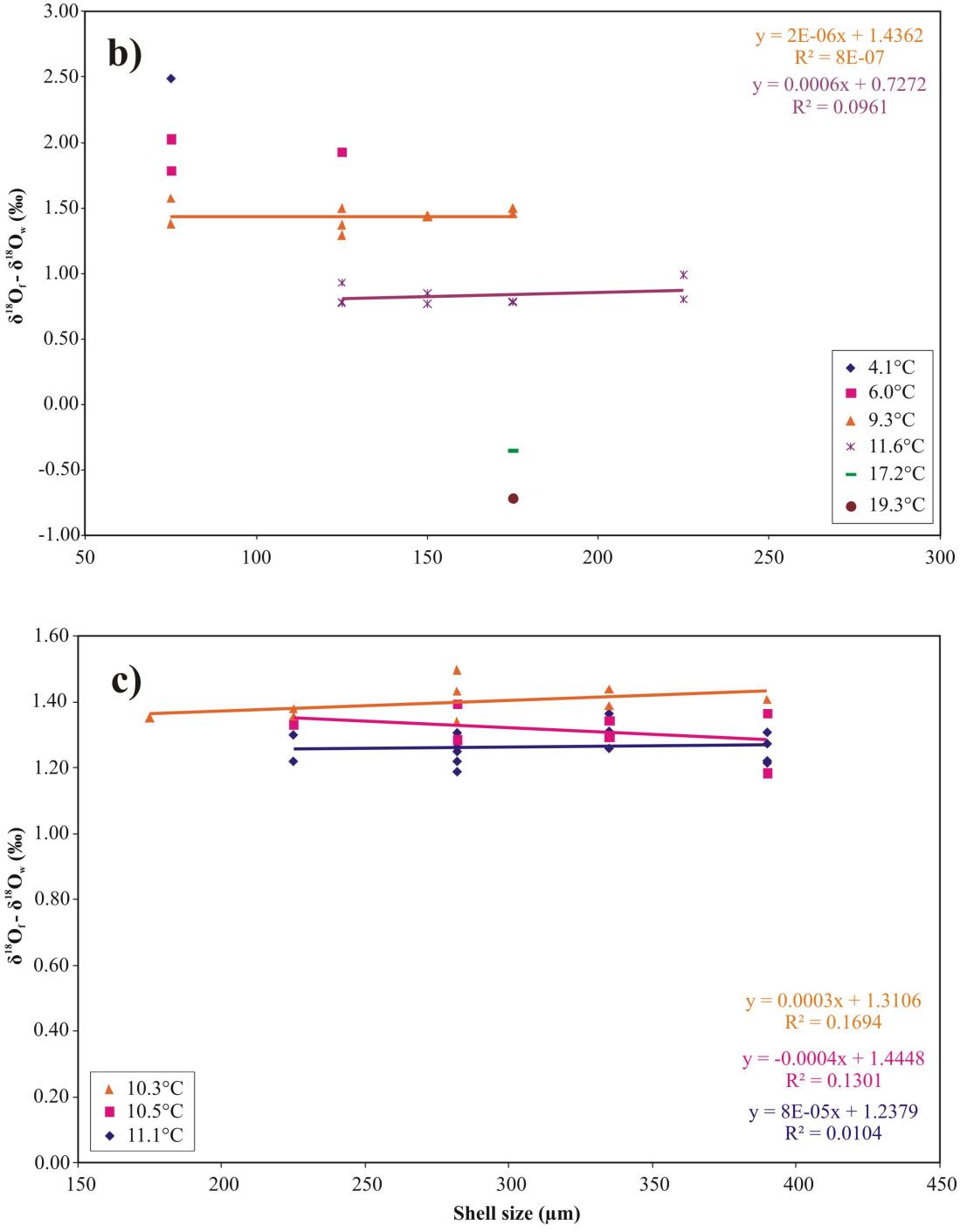


Figure 5.9 (Continued)

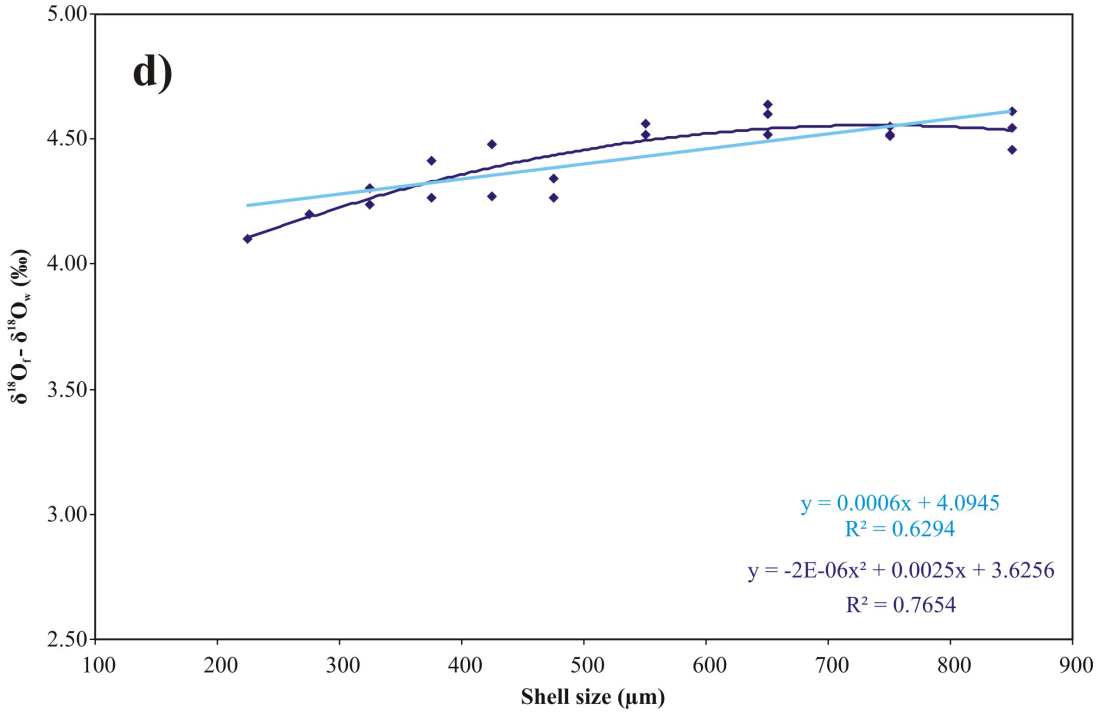


Figure 5.9 (Continued)

The oxygen isotopic composition of foraminiferal shells becomes heavier with increasing size for the system PD+CSI and for the fossils specimens with determination coefficients (R^2) between 0.3 and 0.8. The $\delta^{18}O$:average size relationship for PD+CSI and for fossils record is characterised by regression lines with slopes comprised between 0.0006 (fossil specimens) and 0.002 (from cultured specimens from PD+CSI at 12.7°C). Considering the $\delta^{18}O$ values of specimens calcified in system CSII and *in situ*, there is no clear correlation with test size (low R^2) whatever the temperature considered. The regression lines are almost parallel between the different temperatures in culture where all foraminifera calcified in stable conditions (Figure 5.9 a and b).

For the fossils measurements, the determination coefficient between $\delta^{18}O_f$-$\delta^{18}O_w$ and the size is higher (0.76) when a polynomial regression is applied to the data than with the linear regression (0.63) (Figure 5.9 d). It appears that the oxygen isotopic composition of the chambers does not vary ontogenetically beyond the size of 550 µm.

3.5.2. $\delta^{13}C$ versus size

The $\delta^{13}C_{DIC}$ composition of the seawater was not controlled during our culture experiments. However, we can study the ontogenetic effect on the $\delta^{13}C$ composition of specimens of *B. marginata* that calcified in the same experiment bottles (for CSI and CSII) or Petri dishes (for PD). Figure 5.10 a-b-c present the $\delta^{13}C$ of foraminiferal shells in function of the average size of the foraminifera at the different experimental temperatures for PD, CSI and CSII, respectively. We also represented the influence of the size on the $\delta^{13}C$ composition of *B. marginata* from the Bay of Biscay (Figure 5.10 d) and from fossil samples (Figure 5.10 e).

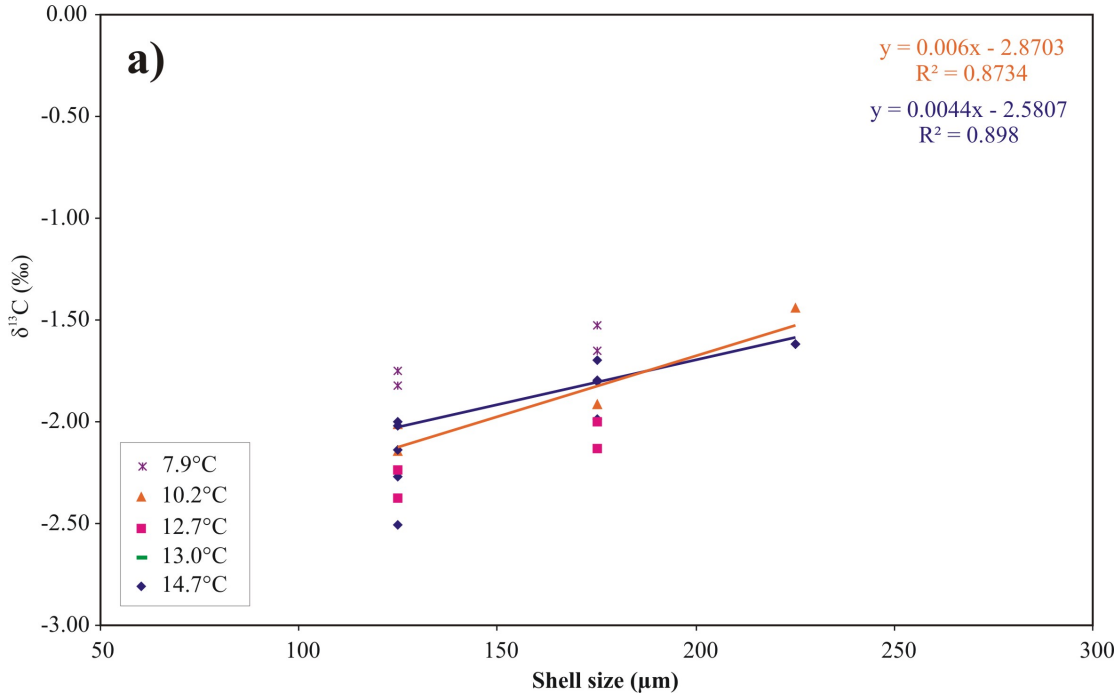


Figure 5.10: Shell size effect on the carbon isotopic composition ($\delta^{13}C$) of B. marginata *from (a) PD experiments, (b) CSI experiments, (c) CSII experiments, (d) Bay of Biscay samples, and (e) fossil samples. Note the different scales in the figures.*

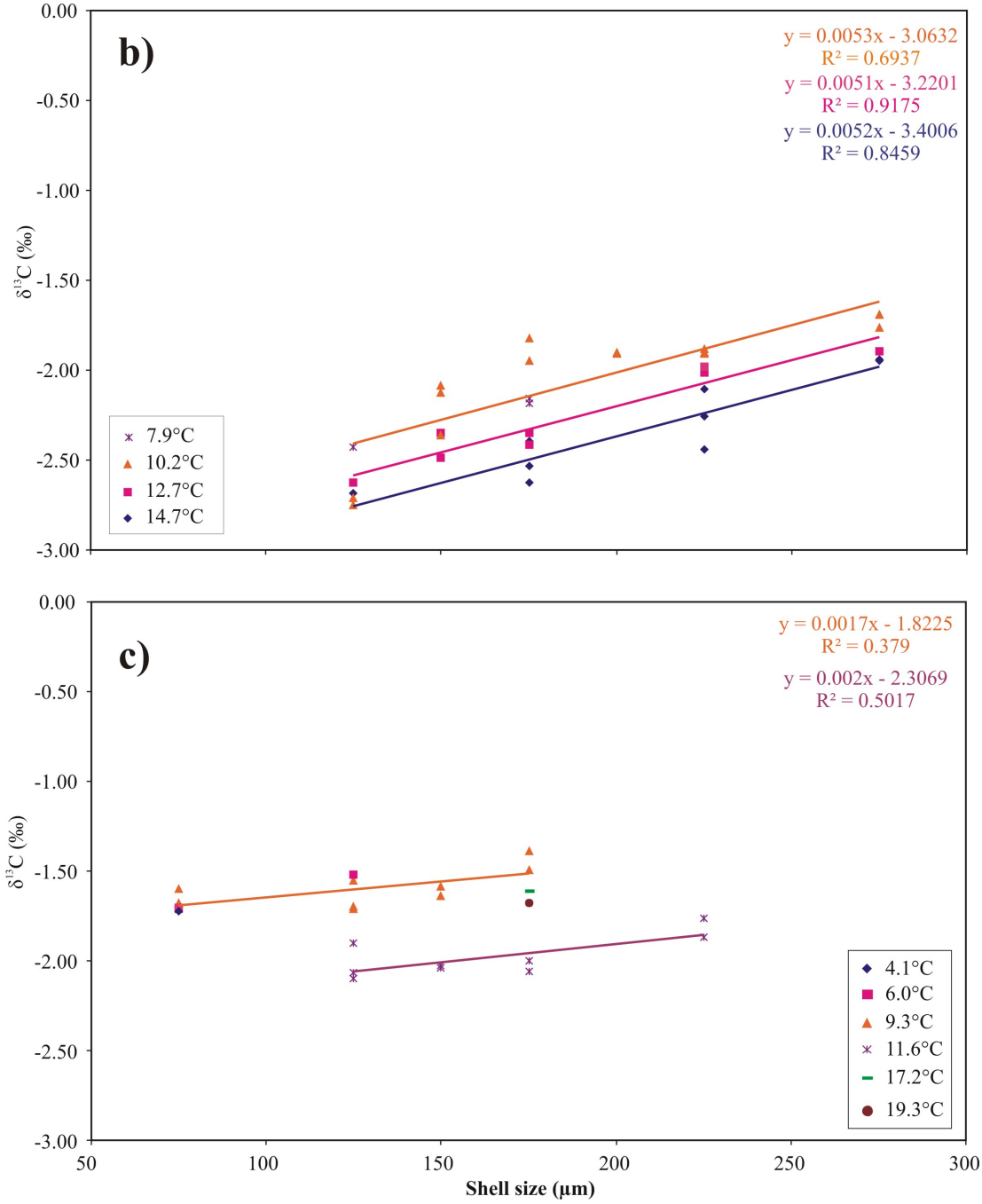


Figure 5.10 (Continued)

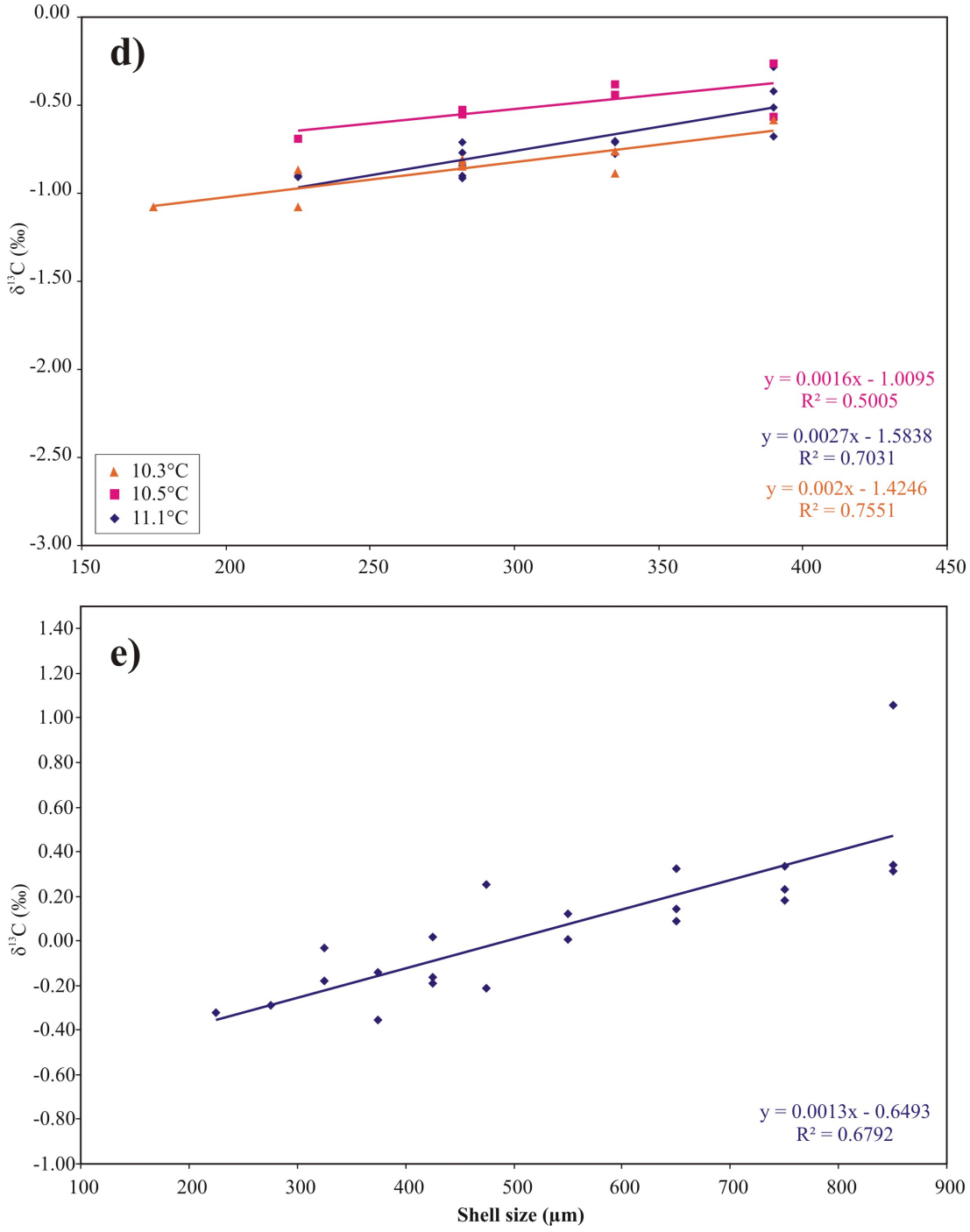


Figure 5.10 (Continued)

The influence of the size on the carbon isotopic composition of *B. marginata* shows the same trend as for $\delta^{18}O$ value with more depleted $\delta^{13}C$ values for smaller specimens and higher $\delta^{13}C$ for larger specimens (Figure 5.10). However, the isotopic fractionation according to size fractions is more important for carbon isotopes than for oxygen isotopes. In all conditions

where enough measurements were available, the regression lines obtained have a slope steeper than for $\delta^{18}O$ with values between 0.0014 and 0.0046.

4. DISCUSSION

4.1. $\delta^{18}O$ composition of cultured foraminifera

During the culture experiments, we succeeded to avoid evaporation of seawater in the systems CSI, CSII and PD. Consequently, the conditions of salinity (and $\delta^{18}O_{seawater}$) were very stable (see Chapter 4 on the experimental protocols) and the $\delta^{18}O$ composition of the foraminifera should only record the different temperature conditions tested in the different experiments. Moreover, we measured the same salinity and $\delta^{18}O_{seawater}$ values in the different systems so that we can compare the isotopic composition of foraminifera between the systems. The carbonate chemistry in the different experiments was more difficult to maintain stable. There was a peak of high alkalinity and pH at the beginning of the PD systems and the pH decreased regularly by 0.3 during the CSII experiments. Considering the absolute values of pH and alkalinity, there were similar conditions in CSI and PD experiments. The alkalinity in the CSII experiments was higher than in the other experiments (CSI and PD), but this difference very probably results from different protocols used to titrate the alkalinity (see Chapter 4). Therefore, the isotopic composition of *B. marginata* calcified in the controlled conditions of all culture experiments can be directly compared.

For a given temperature and a given size fraction, the $^{18}O/^{16}O$ ratios of the shells of *B. marginata* are very similar between the different systems (Figure 5.3). This observation tends to prove that the small variations recorded in the carbonate chemistry in CSII and PD had only minor influence on the $\delta^{18}O$ fractionation of foraminifera. In other words, the isotopic composition of foraminifera is independent of the protocol applied. For both types of systems (closed system and Petri dish system), we succeeded to maintain stable conditions which led to meaningful isotopic composition data for *B. marginata*. These results prove that the protocols presented in Chapter 4 are well suited to perform calibration experiments.

4.2. Calibration equations ($\delta^{18}O_f$-$\delta^{18}O_w$ vs T°C) from culture experiments

Calibration equations ($\delta^{18}O_f$-$\delta^{18}O_w$ versus temperature) were obtained in different size fractions for specimens of *B. marginata* that grew their entire tests in experimental conditions (strategy 1) (Figure 5.4). For the ≤150, 150-200 and 200-250 µm size fractions, the relative influence of the temperature is similar (the slopes of the equations are equal within the range of the standard errors) and independent of the size considered. On the other hand, there is a shift toward more enriched $^{18}O/^{16}O$ ratios with even larger size fractions.

For the > 250 µm size fraction, the linear regression between $\delta^{18}O_f$-$\delta^{18}O_w$ and temperature presents a higher slope compared to the other equations. However, this slope is determined over a small temperature range and is therefore more sensitive to minor experimental errors. In fact, we scarcely obtained juveniles born in the culture experiments that calcified enough to reach a size superior to 250 µm. It was only possible to perform isotopic measurements on specimens > 250 µm at 10.1, 12.7 and 14.7°C. Therefore, a small shift at one temperature could change the slope significantly.

This size fraction > 250 µm has no upper limit. Previously, we saw that the size of the foraminifera has an impact on their $\delta^{18}O$ fractionation. If specimens from the higher temperature had reached a larger size than the specimens from the lower temperature, this could explain their less depleted $\delta^{18}O$ values. However, we calculated the average weight of one specimen (> 250 µm) for the different temperatures and we obtained 3.6, 2.9 and 3.5 µg/specimen respectively for 10.1, 12.7 and 14.7°C. Therefore, there appears not to be a clear increase of the weight (quantity of calcite due to the shell size or the thickness of the walls) with temperature which could explain the shift in the slope of the calibration equation for this size fraction.

It is important to notice that the quantity of foraminifera used for a single isotopic measurement was extremely different between the smallest and the largest size fractions. Between 100 and 200 specimens were required to reach the desired weight for one measurement for specimens <150 µm whereas only 10 to 20 specimens were necessary for the > 250 µm fraction. In fossil samples, 10 specimens is usually a sufficient quantity of foraminifera to obtain a correct $\delta^{18}O$ value. Therefore, more averaged values were obtained for the smaller size fractions than for the larger ones. This could explain part of the difference in slope obtained for the > 250 µm size fraction (Figure 5.4 d), and the fact that we do not see such a shift in the slope of the <150 µm size fraction equation (Figure 5.4 a).

Strategy 2 employed in the culture experiments consisted in introducing specimens of *B. marginata* that had already calcified 2 to 3 chambers in calcein baths at 10°C and then calcified the rest of their tests in controlled conditions at 7.9, 10.2, 12.7 or 14.7°C. We noticed a shift in the slope of the calibration equation of $\delta^{18}O_f$-$\delta^{18}O_w$ according to temperature for these labelled specimens compared to the Shackleton slope (Figure 5.6). All the labelled specimens that grew have been measured before isotopic measurements and they reached average sizes of 205 ± 30, 213 ± 30, 198 ± 27 and 208 ± 29 µm respectively at 7.9, 10.2, 12.7 and 14.7°C. All specimens therefore had the same size range. From 2 to 4 replicate measurements were performed and produced similar data (Figure 5.6).

The labelled specimens of *B. marginata* calcified around 20% of their calcite in calcein baths kept at 10°C. The rest of their tests (80%) were calcified at the different temperatures tested in the physico-chemically controlled experiments. The shift in the slope of the calibration equation for the labelled specimens could come from this artefact: specimens did not precipitate the totality of their test at the same temperature. To check this hypothesis, we calculated what would be the expected $^{18}O/^{16}O$ ratios if 100% of the shells calcified at 7.9, 10.2, 12.7 and 14.7°C (Figure 5.11). The $\delta^{18}O$ composition of foraminifera grown at 10.2°C temperature experiment (PD-10.2) was taken as reference for the calculations ($\delta^{18}O_{\text{theoretical 100\% at 10.2°C}}$ = 1.34‰) and we considered a relation of 0.25‰/°C. We compared this 100% composition to the theoretical $^{18}O/^{16}O$ ratios calculated in the case where 20% of the calcite was formed at 10.2°C and the 80% left were calcified in the different controlled experiments at 7.9, 12.7 and 14.7°C (Figure 5.11). For example, in the case of 80% of shell calcified at 7.9°C and 20% at 10.2%, the calculation to obtain the theoretical composition "80%" is as follow:

$$\delta^{18}O_{\text{theoretical 80\% at 7.9°C}} = 0.80 * \delta^{18}O_{\text{theoretical 100\% at 7.9°C}} + 0.20 * \delta^{18}O_{\text{theoretical 100\% at 10.2°C}}$$

On Figure 5.11, we can see that if 100% of the calcite was calcified at the same temperature, we would observe the same influence of the temperature on the isotopic composition of the foraminifera than for inorganic calcite (slopes of 4 and 4.08 for the theoretical line for 100% of the calcite precipitated at a given temperature and for the Shackleton equation, respectively). Now if we compare the values calculated on the basis that 80% was calcified at the experimental temperatures and 20% was calcified at 10.2°C, we can see that the linear regression plots very close to the one obtained experimentally with labelled specimens. We can conclude that even if the calcite formed before the controlled experiment appeared to be small compared to the quantity of calcite formed in controlled conditions, we measured the

signal of this pre-existing calcite that was precipitated at approximately 10°C (contribution up to 0.3‰ at the highest temperature tested). Therefore, in the case of experiments using labelled small juveniles, it will be necessary in the future to perform laser micro-dissection in order to keep only the part of the shell that was calcified in the desired conditions.

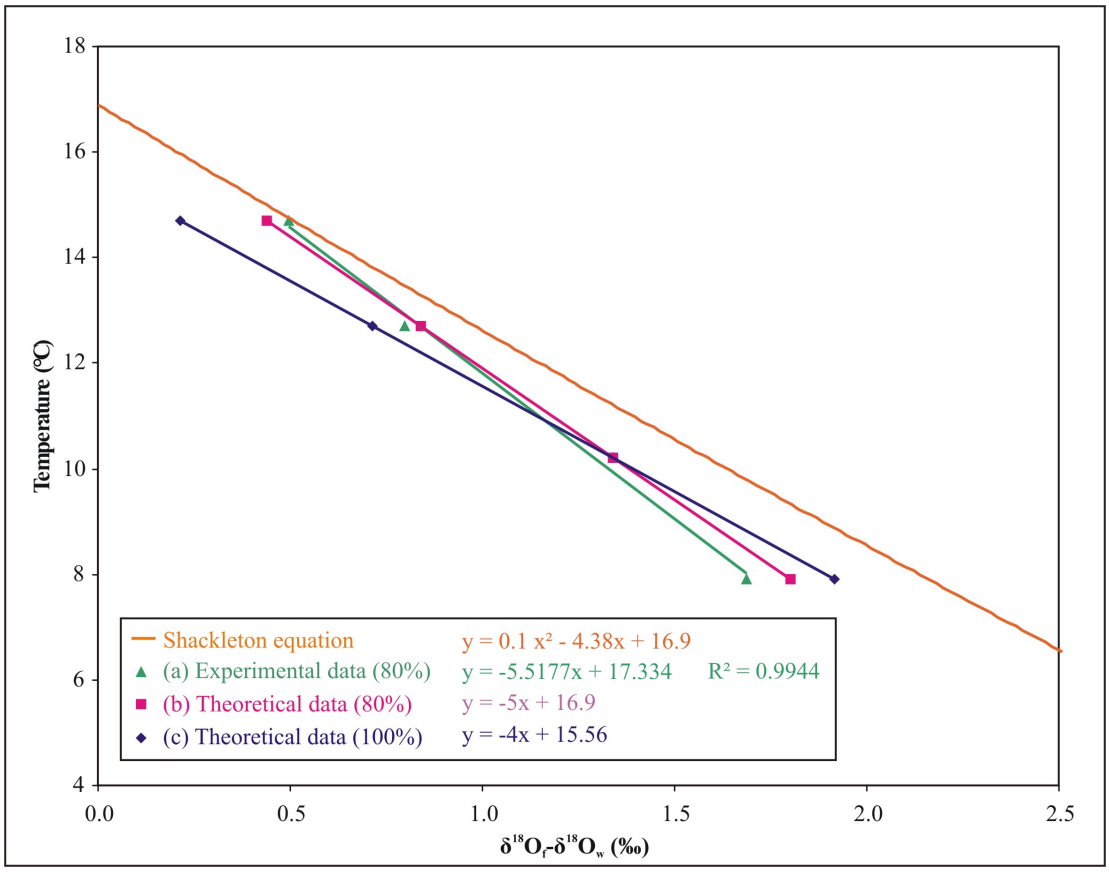


Figure 5.11: Comparison between (a) the experimental $\delta^{18}O_f$-$\delta^{18}O_w$ composition of labelled specimens of B. marginata *that calcified ~80% of their shell in controlled conditions (the remainder of the shell was calcified at 10.2°C), (b) the theoretical composition for the same conditions as for (a) calculated considering a shift of 0.25‰/°C, and (c) the composition of specimens that would have calcified the totality of their shell at the experimental temperatures. The Shackleton equation for equilibrium calcite is indicated. Note the similarity between the experimental (a) and theoretical (b) values.*

4.3. Comparison between calibration equation from culture and field samples and published paleotemperature equations

On Figure 5.12, all the paleotemperature equations determined in this study are reported as well as the paleotemperature equation of Shackleton (1974) adapted from inorganic calcite

(O'Neil, 1969). Considering all size fractions except > 250 µm for culture data, the linear regressions are plotting closer to the theoretical equation with increasing size fractions. We noticed from the fossil data that until the size fraction 500-600 µm, there is an ontogenetic influence on the $\delta^{18}O$ composition of *B. marginata* (Figure 5.9 d). Our specimens obtained in culture experiments reached a maximum size of approximately 300 µm: the larger specimens are isotopically heavier and are closer to the theoretical isotopic equilibrium value. Grossman (1987) measured $\delta^{18}O$ of specimens from the Buliminidae family, *Globobulimina pacifica* and *Loxostomum pseudobeyrichi,* and found out that these species were precipitating close to equilibrium. However, this author did not perform measurements on the genus *Bulimina*.

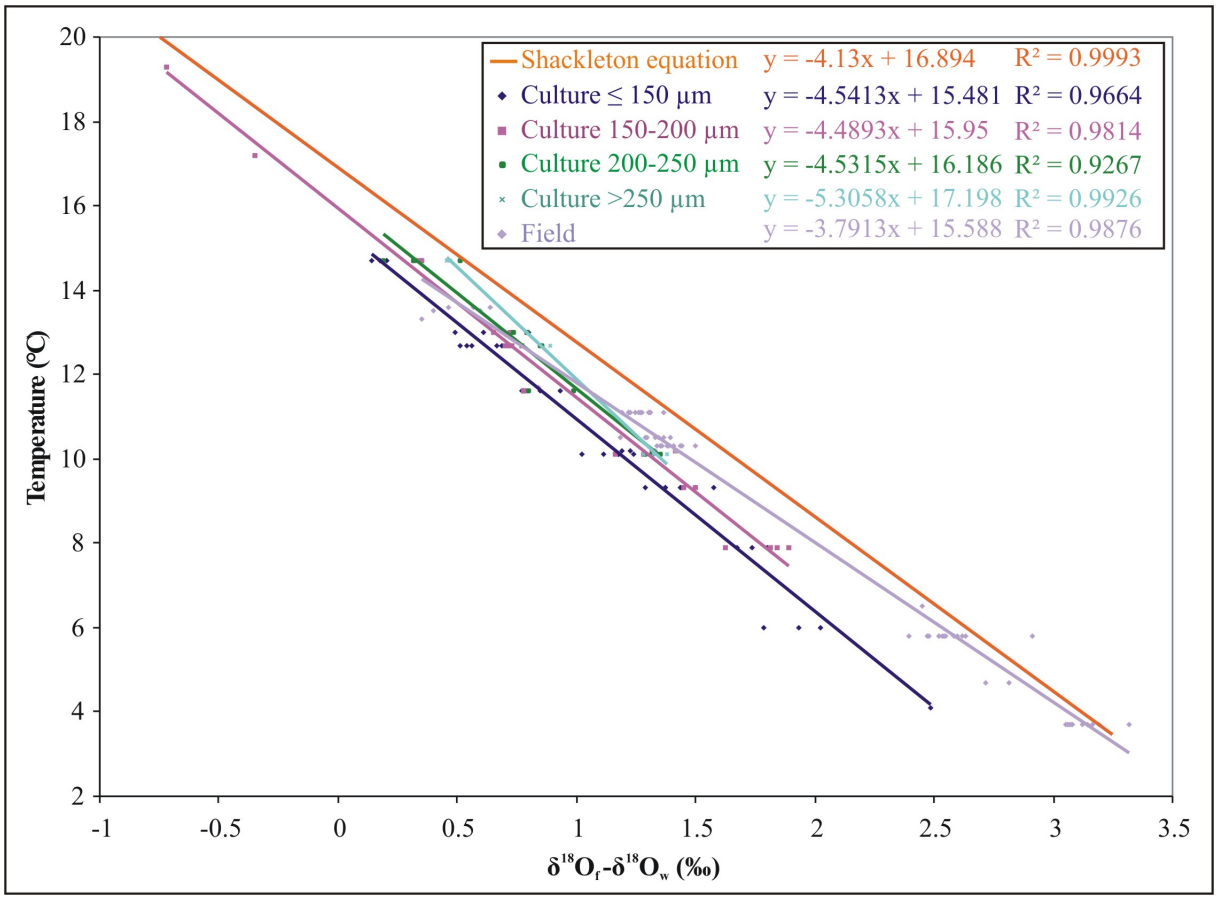


Figure 5.12: Summary of the calibration equations of the oxygen isotopic composition of B. marginata *($\delta^{18}O_f$-$\delta^{18}O_w$) versus temperature obtained from cultures for different size fractions (≤ 150, 150-200, 200-250 and > 250 µm) and from field samples. The Shackleton equation for equilibrium calcite is indicated.*

The fact that pH in the culture experiments was 0.3 units lower (pH ~ 7.9) than in normal seawater pH (pH ~ 8.2) could have an influence on the $\delta^{18}O$ composition of the foraminiferal

calcite. In fact, Zeebe (1999) estimated that a decrease of seawater pH of 0.2-0.3 units would cause an increase of 0.22-0.33‰ in the $\delta^{18}O$ of planktonic foraminiferal calcite (*Orbulina universa*). If we hypothesise that the carbonate ion effect is approximately the same for benthic and planktonic foraminifera, we would expect in our case, of a 0.3 decrease in pH, to obtain enriched $\delta^{18}O$ values for culture *B. marginata* compared to equilibrium or to field data. We observe the opposite so we can deduce that the lower pH values in our experiments are not responsible for the shift observed with the equilibrium equation.

As we explained before, foraminifera produced in culture were smaller than those sampled in natural environments and the isotopic composition of their shells was depleted in ^{18}O compared to bigger specimens. Therefore, if the paleotemperature equations established here from cultured specimens (Figure 5.12) were applied to specimens from sediment samples, the estimated temperature values would be systematically too low. In order to evaluate the error in the estimates, we calculated ΔT as the difference between the theoretical temperature from Shackleton equation (T_{th}) and the temperature estimated with the equations from culture experiments or from field samples without considering core MD77-194 (T_{est}) (Figure 5.13). All the $\delta^{18}O$: temperature relationship established with specimens of *B. marginata* from culture experiments and from field samples predict lower temperatures compared to Shackleton equation (ΔT is always positive). ΔT between the theoretical and experimental temperature estimates is decreasing with increasing temperatures. It should be kept in mind that the estimated temperatures calculated with the equation of the > 250 µm size fraction are not consistent because of the small quantity of data. For the three other size fractions, temperature underestimations relative to Shackleton equation from experimental equations vary from 0.7 to 1.4°C at higher temperatures, and from 1.2 to 2.4°C at lower temperatures. However the shift between the equations of these different size fractions is constant. The equation obtained with specimens sampled in the field produces temperature estimates close to the paleotemperature equation. Temperatures are underestimated between 0.4 and 1°C with this equation relative to the equilibrium equation. The Rhône prodelta samples are responsible for the higher ΔT values observed at the high temperatures which are not characterised by very well define and stable conditions as for the other field area analysed in our study. This can explain why the temperature estimates are worth at higher temperature.

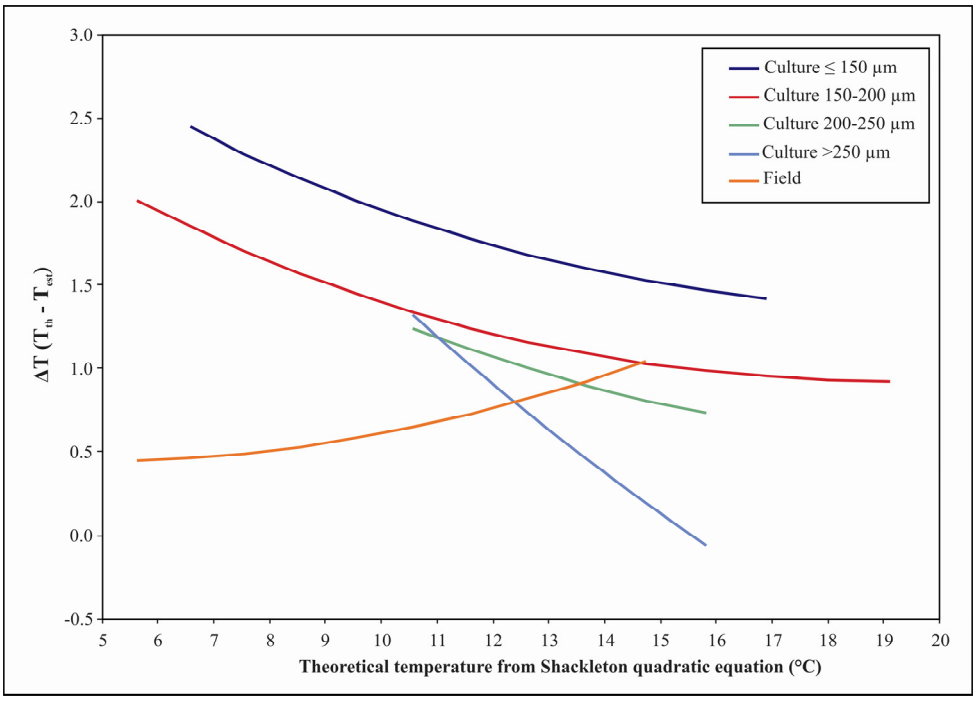


Figure 5.13: Offsets between the theoretical temperature obtained from Shackleton equation (T_{th}) and the temperature calculated from the calibration equation determined in our study (T_{est}): from culture experiments for different size fractions (≤ 150, 150-200, 200-250 and > 250 µm) and from field samples (without MD77-194).

In order to compare in an exhaustive way our calibration equations with the rest of already published paleotemperature equations (McCrea, 1950; Epstein *et al*., 1953; Craig, 1965; Horibe and Oba, 1972; Shackleton, 1974; Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Kim and O'Neil, 1997; Bemis *et al*., 1998), we presented all the regression curves in the range of temperature tested in our study (from around 3 to 20°C) (Figure 5.14, Table 5.5). Our linear regressions are plotting in the scatter of all the equations from the literature. The slopes of all the calibration curves are similar.

At the higher temperatures, the variation depicted by calibration equations of field samples (low slope value) and the > 250 µm size fraction of culture samples (high slope value) increase relative to the scatter of the other equations. The three other culture equations (< 150 µm, 150-200 and 200-250 µm size fractions) exhibit slope values particularly close to the paleotemperature equations obtained experimentally by Kim and O'Neil (1997) for inorganic calcite and by Erez and Luz (1983) and Bouvier-Soumagnac and Duplessy (1985) for planktonic foraminifera (*G. sacculifer* and *O. universa*, respectively). Since the < 150 µm size fraction is not often used in fossil studies, we can conclude that *B. marginata* is a species that could be used by paleoceanographs to reconstruct paleotemperatures using the calibration

equations for the 150-200 or 200-250 µm size fractions, once the effect of the size is taken into account. It appears important for paleoceanographic studies to work with a constant and narrow range of foraminiferal size to achieve a better precision in the reconstructions and limit the error induced by ontogenetic effects.

References	Sources	a	b	c
McCrea (1950)	Inorganic calcite	16.0	-5.17	0.092
Epstein *et al*. (1953)	Mollusks	16.5	-4.3	0.14
Craig (1965)	Mollusks (modified from Epstein, 1953)	16.9	-4.2	0.13
Shackleton (1974)	Modified from O'Neil *et al*. (1969) for inorganic calcite	16.9	-4.38	0.10
Horibe and Oba (1972)	Mollusks	17.04	-4.34	0.16
Shackleton (1974)	Benthic forams (modified from O'Neil *et al*. (1969) for *Uvigerina*)	16.90	-4.00	
Erez and Luz (1983)	Planktonic forams (laboratory, *G. succulifer*)	17.0	-4.50	0.03
Bouvier-Soumagnac and Duplessy (1985)	Planktonic forams (laboratory, *O. universa*)	16.40	-4.67	
	Planktonic forams (Indian ocean, *O. universa*)	15.40	-4.81	
Kim and O'Neil (1997)	Inorganic calcite	16.10	-4.64	0.09
Bemis *et al*. (1998)	Planktonic forams (laboratory, *O. universa,* LL)	16.50	-4.80	
	Planktonic forams (laboratory, *O. universa,* HL)	14.90	-4.80	
	Planktonic forams (laboratory, *G. bulloides,* 11-chambers shell)	12.60	-5.07	
	Planktonic forams (laboratory, *G. bulloides,* 12-chambers shell)	13.20	-4.89	
	Planktonic forams (laboratory, *G. bulloides,* 13-chambers shell)	13.60	-4.77	
Our study	Benthic forams (laboratory, *B. marginata,* < and = 150 µm)	15.48	-4.54	
	Benthic forams (laboratory, *B. marginata,* 150-200 µm)	15.95	-4.49	
	Benthic forams (laboratory, *B. marginata,* 200-250 µm)	16.23	-4.61	
	Benthic forams (laboratory, *B. marginata,* > 250 µm)	17.20	-5.31	
	Benthic forams (laboratory, *B. marginata,* strategy 2)	17.27	-5.42	
	Benthic forams (in situ, *B. marginata,* all sizes)	15.59	-3.79	

Table 5.5: Comparison of commonly used paleotemperature equations with equations developed in our study. The coefficients a, b and c are constants of the equation $T(°C) = a + b\,(\delta c - \delta w) + c\,(\delta c - \delta w)^2$. *LL and HL are the abbreviations for low light and high light, respectively.*

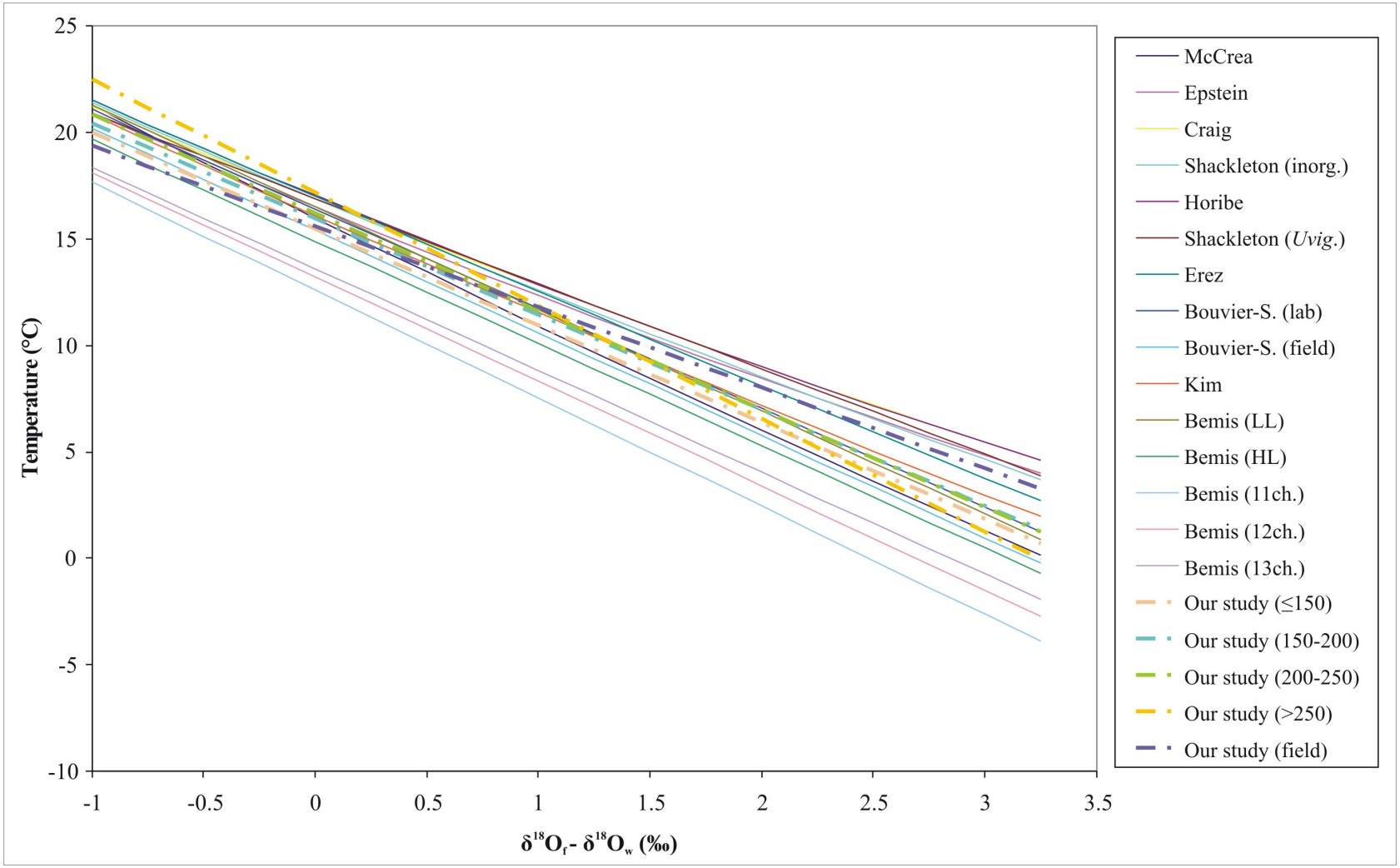


Figure 5.14: Comparison of the temperature predictions using the calibration equations established in our study (from culture experiments for four different size fractions (≤ 150, 150-200, 200-250 and > 250 µm) and from field samples) and published paleotemperature equations.

4.4. Vital effect

The offset often observed between the isotopic composition of foraminiferal calcite and the equilibrium calcite is called the vital effect (Urey *et al*., 1951; Duplessy *et al*., 1970). This isotopic disequilibrium may be explained by two main processes: metabolic and kinetic isotope effects described by McConnaughey (1989a, 1989b) and studied by several authors (e.g. Vinot-Bertouille and Duplessy, 1973; Erez, 1978; Woodruff *et al*., 1980; Grossman, 1987; Bijma *et al*., 1998). The kinetic effect corresponds to the discrimination against heavy isotope of C and O during the hydration ($CO_2 + H_2O \rightarrow H_2CO_3$) and hydroxylation ($CO_2 + OH^- \rightarrow HCO_3^-$) of CO_2. The exchange during hydration and hydroxylation of CO_2 are slower than the precipitation of CO_3^{2-} (McConnaughey, 1989a, 1989b). Therefore the calcification rate plays an important role in the fractionation of the organisms since higher growth rates will result in more depleted composition in $\delta^{18}O$ and $\delta^{13}C$ (McConnaughey, 1989a, 1989b). The metabolic effects are caused by respiration and photosynthesis. These metabolic activities of the organisms have an opposite influence: respiration will decrease shell $\delta^{13}C$ by adding light ^{12}C into the environment where the $CaCO_3$ precipitates, whereas photosynthesis will increase shell $\delta^{13}C$ by using preferentially isotopically light CO_2 (McConnaughey, 1989a). In our case, photosynthesis has no influence since we are working with deep-sea benthic foraminifera (non symbiont bearing) and our experiments took place in the dark.

4.4.1. Ontogenetic effect

Several authors studied the ontogenetic effect on the isotopic composition of planktonic (Berger *et al*., 1978; Oppo and Fairbanks, 1989; Spero and Lea, 1996; Bemis *et al*., 1998; Bijma *et al*., 1998; Elderfield *et al*., 2002) and benthic foraminifera (Vincent *et al*., 1981; Dunbar and Wefer, 1984; Corliss *et al*., 2002; Saraswati, 2004; Schmiedl *et al*., 2004). When sufficient measurements were performed in our study, it was possible to look at the influence of different size fractions on the isotopic composition of *Bulimina marginata* foraminiferal shells (1) calcified in our controlled laboratory experiments, (2) from field samples and (3) from fossil samples (Figures 5.9 and 5.10).

The oxygen and carbon isotopic composition of *B. marginata* shells becomes slightly heavier with increasing size fraction (Figures 5.9, 5.10). The ontogenetic influence on $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios appears higher in the culture experiments PD and CSI than for the *in situ*

specimens. In the culture experiments, specimens are probably growing faster due to the presence of a larger quantity of fresh organic matter in comparison to the food conditions found usually in the sediment. The faster growth could increase the kinetic effect on the fractionation of foraminifera (McConnaughey, 1989a, 1989b). Surprisingly, the results from CSII are different from the other culture systems and there is no apparent influence of the size fraction on the isotopic composition. We do not find any plausible explanation to interpret these results.

Wefer and Killingley (1980) observed lower $\delta^{13}C$ when growth rates were faster in the aragonitic gastropods. Several studies reported enrichment in heavy isotopes with increasing size for different species of planktonic foraminifera (Spero and Lea, 1996; Bijma *et al*., 1998; Elderfield *et al*., 2002). Spero and Lea (1996) reported a higher ontogenetic effect on the oxygen isotopic composition of the planktonic foraminifera *Globigerina bulloides* than the one measured for the benthic species *B. marginata*. These authors also observed an equal size effect on the isotopic composition of *G. bulloides* for both temperatures tested in their experiments (16 and 22°C). Our culture data show regression lines of $\delta^{18}O_f$-$\delta^{18}O_w$ versus size which are relatively parallel between the different temperatures (Figure 5.9 a and b). Therefore, the mechanism responsible for this ontogenetic effect appears to be independent from the calcification temperature.

Previous field-based studies of size-dependent trends in benthic foraminiferal isotopic values have been inconclusive. Generally, benthic foraminifera do not show a significant change in their stable isotope composition with size (Vincent *et al*., 1981; Grossman, 1987; Corliss *et al*., 2002). Dunbar and Wefer (1984) checked the influence of size on 9 species of deep-sea benthic foraminifera and found virtually no influence of size on their isotopic composition, with the possible but doubtful exception of *Uvigerina*. They concluded that the size effect on oxygen and carbon isotopic composition of foraminifera is species specific. More recently, Schmiedl *et al*. (2004) studied the ontogenetic effect on the isotopic fractionation of *Uvigerina mediterranea*. They measured an enrichment of 0.3‰ in $\delta^{18}O$ and around 1‰ in $\delta^{13}C$ over a total size range of 175 to 1250 µm. This enrichment was particularly strong in the younger stages (100–300 µm) and became weaker in the later growth stages. They observe a logarithmic shape of the relation between isotopic composition and foraminiferal size, just like the one we observed in the $\delta^{18}O$ fossil record. In our dataset for the fossil foraminifera, the $^{18}O/^{16}O$ ratio is no longer influenced by size beyond a threshold of about 550 µm (Figure 5.9 d). However, this limit is not observed in the $\delta^{13}C$ data (Figure 5.10 e). Spero and Lea (1996) observed this threshold in ontogenetic effect only for the $\delta^{13}C$ composition of *G.*

bulloides. Equilibrium isotope partitioning is observed when $CaCO_3$ precipitates slowly from solution (McConnaughey, 1989b). Then bigger specimens may reach the equilibrium because the calcification is slower.

4.4.2. $\delta^{18}O$ vs. $\delta^{13}C$

There is a vital effect on both oxygen and carbon isotopic composition of foraminiferal shell. For some experimental data, we observe linear correlations between $\delta^{13}C$ and $\delta^{18}O$ values of foraminifera that calcified in a same culture experiment (one given culture system and one given temperature) and had different sizes (Figure 5.15). This is the case of experiments PD-12.7, PD-14.7, CSI-12.7 and CSII-11.6 which exhibit linear covariance with slopes between 0.35 and 0.56 (Figure 5.15). For the other experiments, either not enough measurements were available or the coefficients of determination was lower than 0.6. The kinetic effect could be responsible for the observed covariance between oxygen and carbon isotopic fractionation (McConnaughey, 1989a). The fact that younger foraminifera calcify faster (Berger *et al*., 1978) would not leave the time to obtain equilibrium in the calcification reservoir before crystallisation and would result in the production of more negative $\delta^{18}O$ and $\delta^{13}C$ values. This phenomenon was already proposed by Turner (1982). For benthic foraminifera, ecological experiments tend to prove that growth of specimens is not uniform and chambers addition is faster during the first ontogenetic stages (Bradshaw, 1957, 1961; Hemleben and Kitazato, 1995; Stouff *et al*., 1999a) and in particular for *Bulimina marginata* (Barras *et al*., submitted). Then kinetic effect is a consequence of incomplete isotopic equilibration which should lead to a linear correlation between $\delta^{13}C$ and $\delta^{18}O$. Linear correlations are not observed in all culture experiment perhaps because of the lack of data or it is possible that another effect is involved in the disequilibrium observed.

Grossman (1987) estimated that the so-called vital effect has a greater influence on $\delta^{13}C$ than on $\delta^{18}O$, which corresponds to our observations in our experiments. The fact that the $\delta^{13}C$ is more influence by the size of the foraminifera could also be explained by an increased respiration rate when specimens are younger (Berger *et al*., 1978). Respiration lowers the $\delta^{13}C$ of the inorganic carbon reservoir because biomass is relatively depleted in ^{13}C and the organisms would incorporate more depleted metabolic CO_2 (McConnaughey, 1989a).

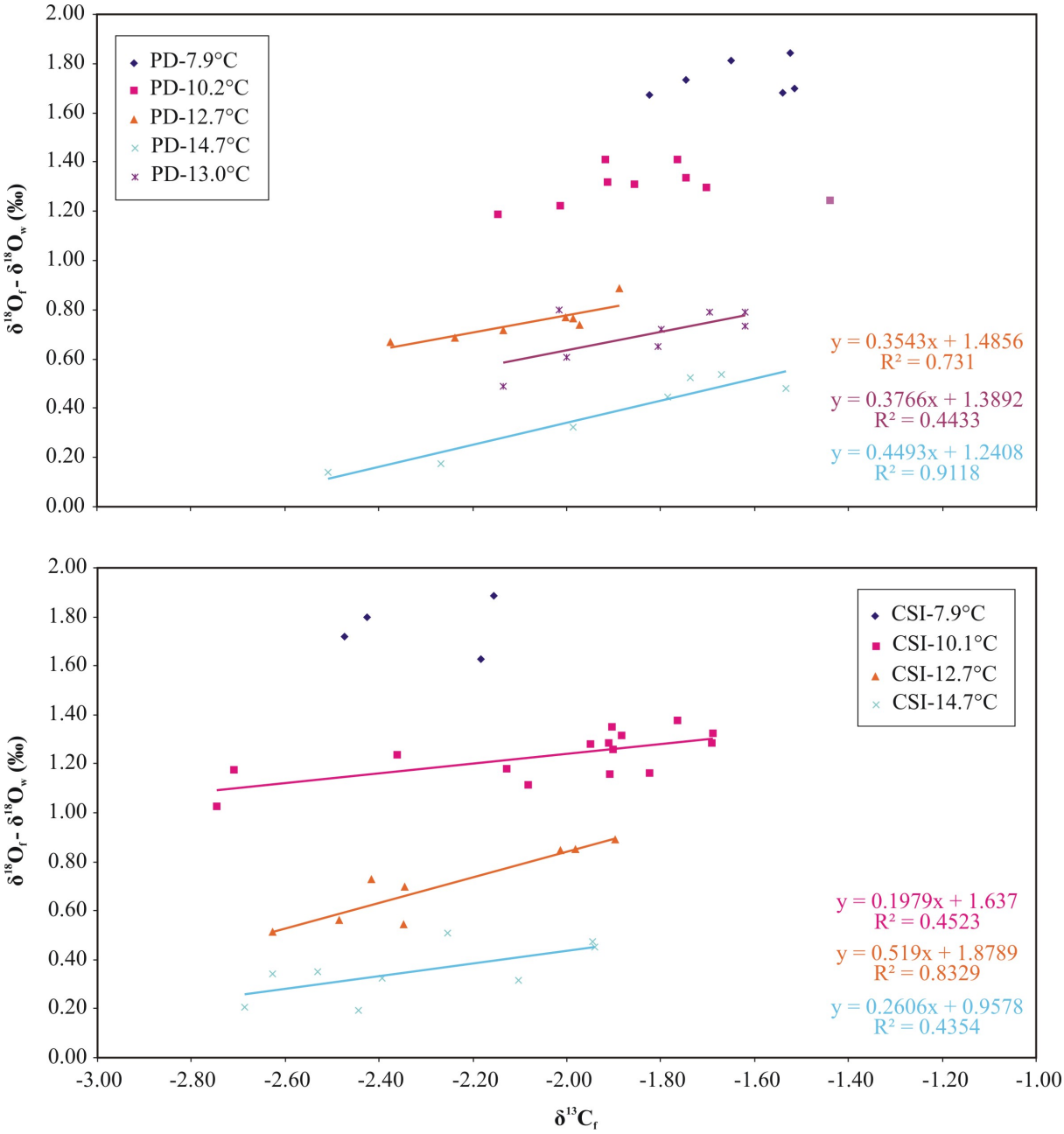


Figure 5.15: $\delta^{13}C$ versus $\delta^{18}O$ (corrected by the oxygen isotopic composition of the seawater) for specimens of B. marginata *that calcified their entire test in (a) PD experiments, (b) CSI experiments, and (c) in CSII experiments.*

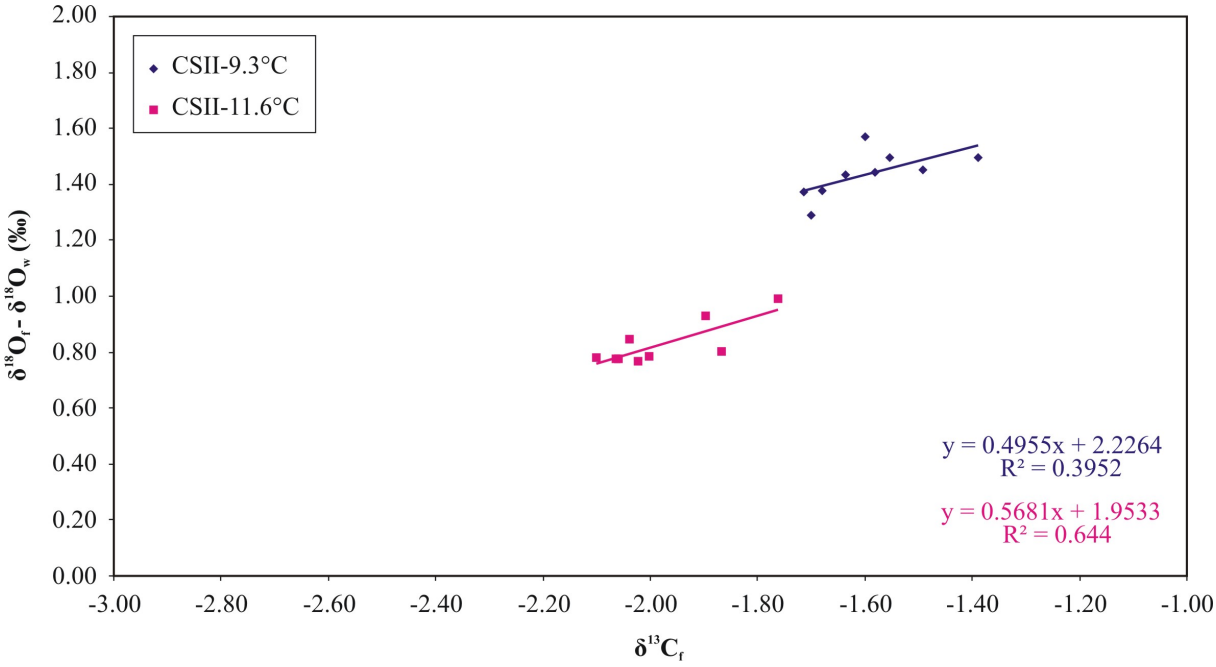


Figure 5.15 (Continued)

4.5. $\delta^{13}C$ and temperature

From figures representing the $\delta^{13}C$ of foraminifera in function of the different size fractions (Figure 5.10 a-b-c), we can observe that there seems to be an impact of the different temperature conditions in the culture experiments. In the CSI data, there is a clear shift in $\delta^{13}C$ values toward more depleted composition with increasing temperature, just as for $\delta^{18}O$ values. This trend is not observed for the *in situ* specimens. The most obvious explanation for this apparent influence of temperature on the $\delta^{13}C$ composition of *B. marginata* is that, at higher temperatures, the organic matter degradation by bacterial activity could be much higher than at lower temperatures. Higher oxidation of the organic matter induces production of $\delta^{13}C$ depleted CO_2 which could, in case it was used for the construction of the shell, result in more depleted $\delta^{13}C$ of the foraminiferal shells. As was proposed by Spero *et al*. (1991), the physiological rate is also increasing with temperature which means that there is an increase in the respiration and/or calcification rates. The increase in respiration rate would result in more ^{12}C release and consequently, in a relative $\delta^{13}C$ depletion in the ambient seawater which could also cause more depleted isotopic composition at higher temperature. Moreover, the increase in calcification rate would increase the fractionation kinetic effect so that the foraminiferal carbon isotopic composition would be even more negative at higher temperature.

The presence of a feeding cyst around the specimens of *B. marginata* (as reported in the Chapter 3) could create particular microenvironmental conditions within this cyst. The

geochemical composition of the ambient seawater could differ from the conditions inside this microenvironment. The seawater in this microenvironment should be depleted in $\delta^{13}C$ compared to overlying seawater in the rest of the experiment due to the release of ^{12}C during the oxygenation of the fresh organic matter constituting the cyst. Therefore, the presence of the feeding cyst could explain why *B. marginata* exhibit such a depleted $\delta^{13}C$ in our experiments. However, in the absence of data on the $\delta^{13}C_{DIC}$ of the seawater in our cultures, it is difficult to give more conclusive interpretation.

5. CONCLUSION

Several important conclusions arise from this culture and *in situ* study of the influence of different calcification temperatures on the oxygen isotopic composition of *B. marginata*. We developed calibration equations for specimens of *B. marginata* that calcified the totality of their shell in controlled laboratory conditions. We observed that there is a small but significant ontogenetic effect on the $\delta^{18}O$ composition of this species so that different equations must be established for specific shell sizes:

$$T\,(°C) = 15.48\,(\pm 0.18) - 4.54\,(\pm 0.14) * (\delta^{18}O_f - \delta^{18}O_w) \quad R^2 = 0.97$$
for the ≤150 µm size fraction (1)

$$T\,(°C) = 15.95\,(\pm 0.14) - 4.49\,(\pm 0.13) * (\delta^{18}O_f - \delta^{18}O_w) \quad R^2 = 0.98$$
for the 150-200 µm size fraction (2)

$$T\,(°C) = 16.23\,(\pm 0.35) - 4.61\,(\pm 0.37) * (\delta^{18}O_f - \delta^{18}O_w) \quad R^2 = 0.93$$
for the 200-250 µm size fraction (3)

$$T\,(°C) = 17.20\,(\pm 0.24) - 5.31\,(\pm 0.23) * (\delta^{18}O_f - \delta^{18}O_w) \quad R^2 = 0.99$$
for the >250 µm size fraction (4)

The <150 µm size fraction is usually not analysed in paleoceanographic studies and the equation for the >250 µm size fraction is probably biased by the low amount of data. Equations (1) and (4) are therefore not well adapted for paleoceanographic reconstructions. For future studies were fossil specimens of *B. marginata* could be analysed, we would therefore recommend, when large specimens are not available, to use equations (2) or (3), depending on the size fraction analysed. These two equations have been established for a temperature range of 7.9-19.3°C for equation (2) and 10.1-14.7°C for equation (3). To be in

agreement with the oxygen isotopic equilibrium values predicted by the paleotemperature equation of Shackleton (1974), it is however necessary to apply a correction factor to the oxygen isotopic values of the specimens of *B. marginata* in order to take into account the offset due to vital effect. The average correction factor is +0.27‰ for the 150-200 µm size fraction (equation 2) and +0.24‰ for the 200-250 µm size fraction (equation 3). According to these relations, a change in calcification temperature of 1°C alters the $\delta^{18}O$ value of foraminiferal shell by 0.22‰.

The equations established with experimentally produced specimens of *B. marginata* fit well with the *in situ* paleotemperature equation (without considering the data of core MD77-194):

$$T\ (°C) = 15.59\ (\pm\ 0.11) - 3.79\ (\pm\ 0.05) * (\delta^{18}O_f - \delta^{18}O_w) \qquad R^2 = 0.99$$

for *in situ* samples (5)

However, it is important to keep in mind that the high temperature data defining this calibration equation were sampled at shallow water depth where physico-chemical conditions (e.g. temperature and salinity) were probably less stable than in deep sea ocean. It would be interesting to analyse specimens of *B. marginata* that calcified in deep sea environments where the temperature is high (superior to 12°C) in order to further precise this field calibration.

In this study, we also analysed the oxygen isotopic composition of specimens of *B. marginata* that calcified about 20% of their shell previously to their introduction in controlled experiments so that about 80% of their shell calcified in the desired experimental conditions. The $\delta^{18}O$ composition of the entire shells clearly shows that the signal corresponding to the pre-existing calcite has a significant influence. Our conclusion is that a more precise analytical method, e.g. microlaser dissection of the calcite formed previously to controlled conditions, is primordial in the case of experiments where foraminifera were not born and calcified their entire shell in the desired culture experiments.

Finally, this study proved that culture experiments in controlled conditions with deep-sea benthic foraminifera are leading to exploitable results so that culture experiments in laboratory are definitely an interesting way to study in detail the impact of individual physico-chemical parameters (temperature, salinity, carbonate chemistry…) on benthic foraminiferal shell chemistry (isotopes, trace metals…).

***Appendix 5.1** : Oxygen and carbon isotopic data of* Bulimina marginata (marginata *and* aculeata *morphotypes) from culture experiments (PD, CSI and CSII) and from the field (Bay of Biscay, Rhône prodelta, Cape Blanc and Indian Ocean).*

Experiment / Station	Temp. (°C)	Morphotype	Size fraction	Strategy / depth in sediment (cm)	$\delta^{18}O$ (‰PDB)	$\delta^{13}C$ (‰PDB)	$\delta^{18}O_w$ (‰SMOW)	$\delta^{18}O_f$-$\delta^{18}O_w$ (‰PDB)
Petri dish system PD								
PD-7.9	7.9	*marginata*	< 150	1	**2.02**	**-1.75**	0.55	**1.74**
PD-7.9	7.9	*marginata*	< 150	1	**1.95**	**-1.82**	0.55	**1.67**
PD-7.9	7.9	*marginata*	150-200	1	**2.09**	**-1.65**	0.55	**1.81**
PD-7.9	7.9	*marginata*	150-200	1	**2.12**	**-1.52**	0.55	**1.84**
PD-7.9	7.9	*marginata*	-	2	**1.98**	**-1.52**	0.55	**1.70**
PD-7.9	7.9	*marginata*	-	2	**1.96**	**-1.54**	0.55	**1.68**
PD-10.2	10.2	*marginata*	< 150	1	**1.47**	**-2.15**	0.55	**1.19**
PD-10.2	10.2	*marginata*	< 150	1	**1.50**	**-2.01**	0.55	**1.22**
PD-10.2	10.2	*marginata*	150-200	1	**1.60**	**-1.91**	0.55	**1.32**
PD-10.2	10.2	*marginata*	150-200	1	**1.69**	**-1.92**	0.55	**1.41**
PD-10.2	10.2	*marginata*	200-250	1	**1.53**	**-1.44**	0.55	**1.25**
PD-10.2	10.2	*marginata*	-	2	**1.58**	**-1.70**	0.55	**1.30**
PD-10.2	10.2	*marginata*	-	2	**1.69**	**-1.77**	0.55	**1.41**
PD-10.2	10.2	*marginata*	-	2	**1.59**	**-1.86**	0.55	**1.31**
PD-10.2	10.2	*marginata*	-	2	**1.61**	**-1.74**	0.55	**1.33**
PD-12.7	12.7	*marginata*	< 150	1	**0.97**	**-2.24**	0.55	**0.69**
PD-12.7	12.7	*marginata*	< 150	1	**0.95**	**-2.37**	0.55	**0.67**
PD-12.7	12.7	*marginata*	150-200	1	**1.00**	**-2.13**	0.55	**0.72**
PD-12.7	12.7	*marginata*	150-200	1	**1.05**	**-2.00**	0.55	**0.77**
PD-12.7	12.7	*marginata*	-	2	**1.05**	**-1.99**	0.55	**0.77**
PD-12.7	12.7	*marginata*	-	2	**1.02**	**-1.97**	0.55	**0.74**
PD-12.7	12.7	*marginata*	-	2	**1.16**	**-1.89**	0.55	**0.88**
PD-14.7	14.7	*marginata*	< 150	1	**0.46**	**-2.27**	0.55	**0.18**
PD-14.7	14.7	*marginata*	< 150	1	**0.42**	**-2.51**	0.55	**0.14**
PD-14.7	14.7	*marginata*	150-200	1	**0.60**	**-1.99**	0.55	**0.32**
PD-14.7	14.7	*marginata*	-	2	**0.73**	**-1.79**	0.55	**0.45**
PD-14.7	14.7	*marginata*	-	2	**0.76**	**-1.53**	0.55	**0.48**
PD-14.7	14.7	*marginata*	-	2	**0.82**	**-1.67**	0.55	**0.54**
PD-14.7	14.7	*marginata*	-	2	**0.80**	**-1.74**	0.55	**0.52**
PD-15.7	15.7	*aculeata*	150-200	1	**0.31**	**-2.03**	0.55	**0.03**
PD-15.7	15.7	*aculeata*	150-200	1	**0.39**	**-2.04**	0.55	**0.11**
PD-15.7	15.7	*aculeata*	200-250	1	**0.42**	**-1.84**	0.55	**0.14**
PD-13.0	13.0	*marginata*	< 150	1	**0.89**	**-2.00**	0.55	**0.61**
PD-13.0	13.0	*marginata*	< 150	1	**1.08**	**-2.02**	0.55	**0.80**
PD-13.0	13.0	*marginata*	< 150	1	**0.77**	**-2.13**	0.55	**0.49**
PD-13.0	13.0	*marginata*	150-200	1	**0.93**	**-1.81**	0.55	**0.65**
PD-13.0	13.0	*marginata*	150-200	1	**1.00**	**-1.80**	0.55	**0.72**
PD-13.0	13.0	*marginata*	150-200	1	**1.07**	**-1.70**	0.55	**0.79**
PD-13.0	13.0	*marginata*	200-250	1	**1.01**	**-1.62**	0.55	**0.73**
PD-13.0	13.0	*marginata*	200-250	1	**1.07**	**-1.62**	0.55	**0.79**

Appendix 5.1 *(Continued)*

Experiment / Station	Temp. (°C)	Morphotype	Size fraction	Strategy / depth in sediment (cm)	$\delta^{18}O$ (‰ PDB)	$\delta^{13}C$ (‰ PDB)	$\delta^{18}O_w$ (‰ SMOW)	$\delta^{18}O_f$-$\delta^{18}O_w$ (‰ PDB)
Closed system CSI								
CSI-7.9	7.9	*marginata*	-	1	**2.00**	**-2.47**	0.55	**1.72**
CSI-7.9	7.9	*marginata*	< 150	1	**2.08**	**-2.43**	0.55	**1.80**
CSI-7.9	7.9	*marginata*	> 150	1	**1.91**	**-2.18**	0.55	**1.63**
CSI-7.9	7.9	*marginata*	> 150	1	**2.17**	**-2.16**	0.55	**1.89**
CSI-10.1	10.1	*marginata*	< 150	1	**1.31**	**-2.75**	0.55	**1.03**
CSI-10.1	10.1	*marginata*	< 150	1	**1.45**	**-2.71**	0.55	**1.17**
CSI-10.1	10.1	*marginata*	=150	1	**1.39**	**-2.08**	0.55	**1.11**
CSI-10.1	10.1	*marginata*	=150	1	**1.46**	**-2.13**	0.55	**1.18**
CSI-10.1	10.1	*marginata*	=150	1	**1.52**	**-2.36**	0.55	**1.24**
CSI-10.1	10.1	*marginata*	150-200	1	**1.56**	**-1.95**	0.55	**1.28**
CSI-10.1	10.1	*marginata*	150-200	1	**1.44**	**-1.82**	0.55	**1.16**
CSI-10.1	10.1	*marginata*	150-250	1	**1.44**	**-1.91**	0.55	**1.16**
CSI-10.1	10.1	*marginata*	150-250	1	**1.54**	**-1.90**	0.55	**1.26**
CSI-10.1	10.1	*marginata*	200-250	1	**1.60**	**-1.88**	0.55	**1.32**
CSI-10.1	10.1	*marginata*	200-250	1	**1.57**	**-1.91**	0.55	**1.29**
CSI-10.1	10.1	*marginata*	200-250	1	**1.63**	**-1.90**	0.55	**1.35**
CSI-10.1	10.1	*marginata*	> 250	1	**1.61**	**-1.69**	0.55	**1.33**
CSI-10.1	10.1	*marginata*	> 250	1	**1.66**	**-1.76**	0.55	**1.38**
CSI-10.1	10.1	*marginata*	> 250	1	**1.56**	**-1.69**	0.55	**1.28**
CSI-12.7	12.7	*marginata*	< 150	1	**0.79**	**-2.63**	0.55	**0.51**
CSI-12.7	12.7	*marginata*	=150	1	**0.82**	**-2.35**	0.55	**0.54**
CSI-12.7	12.7	*marginata*	=150	1	**0.84**	**-2.49**	0.55	**0.56**
CSI-12.7	12.7	*marginata*	> 150	1	**0.98**	**-2.35**	0.55	**0.70**
CSI-12.7	12.7	*marginata*	150-200	1	**1.01**	**-2.42**	0.55	**0.73**
CSI-12.7	12.7	*marginata*	200-250	1	**1.12**	**-2.01**	0.55	**0.84**
CSI-12.7	12.7	*marginata*	200-250	1	**1.13**	**-1.98**	0.55	**0.85**
CSI-12.7	12.7	*marginata*	> 250	1	**1.17**	**-1.90**	0.55	**0.89**
CSI-12.7	12.7	*aculeata*	-	1	**1.15**	**-1.99**	0.55	**0.87**
CSI-12.7	12.7	*aculeata*	-	1	**1.10**	**-2.17**	0.55	**0.82**
CSI-14.7	14.7	*marginata*	< 150	1	**0.49**	**-2.69**	0.55	**0.21**
CSI-14.7	14.7	*marginata*	150-200	1	**0.61**	**-2.39**	0.55	**0.33**
CSI-14.7	14.7	*marginata*	150-200	1	**0.62**	**-2.63**	0.55	**0.34**
CSI-14.7	14.7	*marginata*	150-200	1	**0.63**	**-2.53**	0.55	**0.35**
CSI-14.7	14.7	*marginata*	200-250	1	**0.60**	**-2.10**	0.55	**0.32**
CSI-14.7	14.7	*marginata*	200-250	1	**0.79**	**-2.25**	0.55	**0.51**
CSI-14.7	14.7	*marginata*	200-250	1	**0.47**	**-2.44**	0.55	**0.19**
CSI-14.7	14.7	*marginata*	> 250	1	**0.73**	**-1.94**	0.55	**0.45**
CSI-14.7	14.7	*marginata*	> 250	1	**0.75**	**-1.95**	0.55	**0.47**
CSI-14.7	14.7	*aculeata*	-	1	**0.53**	**-2.37**	0.55	**0.25**
CSI-14.7	14.7	*aculeata*	-	1	**0.80**	**-2.35**	0.55	**0.52**
CSI-14.7	14.7	*aculeata*	-	1	**0.61**	**-2.52**	0.55	**0.33**
Closed system CSII								
CSII-4.1	4.1	*marginata*	< 100	1	**2.77**	**-1.73**	0.55	**2.49**
CSII-6.0	6.0	*marginata*	< 100	1	**2.31**	**-1.71**	0.55	**2.03**
CSII-6.0	6.0	*marginata*	< 100	1	**2.07**	**-1.70**	0.55	**1.79**
CSII-6.0	6.0	*marginata*	100-150	1	**2.21**	**-1.52**	0.55	**1.93**

Appendix 5.1 *(Continued)*

Experiment / Station	Temp. (°C)	Morphotype	Size fraction	Strategy / depth in sediment (cm)	$\delta^{18}O$ (‰ PDB)	$\delta^{13}C$ (‰ PDB)	$\delta^{18}O_w$ (‰ SMOW)	$\delta^{18}O_f$-$\delta^{18}O_w$ (‰ PDB)
CSII-9.3	9.3	*marginata*	< 100	1	**1.85**	**-1.60**	0.55	**1.57**
CSII-9.3	9.3	*marginata*	< 100	1	**1.66**	**-1.68**	0.55	**1.38**
CSII-9.3	9.3	*marginata*	100-150	1	**1.78**	**-1.55**	0.55	**1.50**
CSII-9.3	9.3	*marginata*	100-150	1	**1.65**	**-1.71**	0.55	**1.37**
CSII-9.3	9.3	*marginata*	100-150	1	**1.57**	**-1.70**	0.55	**1.29**
CSII-9.3	9.3	*marginata*	=150	1	**1.71**	**-1.64**	0.55	**1.43**
CSII-9.3	9.3	*marginata*	=150	1	**1.72**	**-1.58**	0.55	**1.44**
CSII-9.3	9.3	*marginata*	150-200	1	**1.78**	**-1.39**	0.55	**1.50**
CSII-9.3	9.3	*marginata*	150-200	1	**1.73**	**-1.49**	0.55	**1.45**
CSII-11.6	11.6	*marginata*	100-150	1	**1.21**	**-1.90**	0.55	**0.93**
CSII-11.6	11.6	*marginata*	100-150	1	**1.06**	**-2.06**	0.55	**0.78**
CSII-11.6	11.6	*marginata*	100-150	1	**1.06**	**-2.10**	0.55	**0.78**
CSII-11.6	11.6	*marginata*	=150	1	**1.13**	**-2.04**	0.55	**0.85**
CSII-11.6	11.6	*marginata*	=150	1	**1.05**	**-2.02**	0.55	**0.77**
CSII-11.6	11.6	*marginata*	150-200	1	**1.06**	**-2.06**	0.55	**0.78**
CSII-11.6	11.6	*marginata*	150-200	1	**1.07**	**-2.00**	0.55	**0.79**
CSII-11.6	11.6	*marginata*	200-250	1	**1.27**	**-1.76**	0.55	**0.99**
CSII-11.6	11.6	*marginata*	200-250	1	**1.08**	**-1.87**	0.55	**0.80**
CSII-17.2	17.2	*marginata*	150-200	1	**-0.07**	**-1.61**	0.55	**-0.35**
CSII-17.2	17.2	*aculeata*	150-200	1	**0.12**	**-1.64**	0.55	**-0.16**
CSII-17.2	17.2	*aculeata*	150-200	1	**-0.01**	**-1.64**	0.55	**-0.29**
CSII-17.2	17.2	*aculeata*	200-250	1	**-0.02**	**-1.63**	0.55	**-0.30**
CSII-19.3	19.3	*marginata*	150-200	1	**-0.44**	**-1.68**	0.55	**-0.72**
CSII-19.3	19.3	*aculeata*	150-200	1	**-0.25**	**-1.33**	0.55	**-0.53**
CSII-19.3	19.3	*aculeata*	150-200	1	**-0.54**	**-1.51**	0.55	**-0.82**
Bay of Biscay								
OB3D	11.7	*marginata*	-	0 - 1	**1.27**	**-1.14**	0.70	**0.84**
OB9J	10.2	*marginata*	-	2 -10	**1.76**	**-0.49**	0.70	**1.33**
OB9J	10.2	*marginata*	-	2 -10	**1.84**	**-0.42**	0.70	**1.41**
SC1S	10.3	*marginata*	150-200	0 - 0.50	**1.78**	**-1.08**	0.70	**1.35**
SC1S	10.3	*marginata*	200-250	0 - 0.25	**1.81**	**-0.87**	0.70	**1.38**
SC1S	10.3	*marginata*	200-250	0 - 0.25	**1.79**	**-1.08**	0.70	**1.36**
SC1S	10.3	*marginata*	250-315	0 - 0.25	**1.77**	**-0.85**	0.70	**1.34**
SC1S	10.3	*marginata*	250-315	0 - 0.25	**1.86**	**-0.81**	0.70	**1.43**
SC1S	10.3	*marginata*	250-315	0 - 0.25	**1.93**	**-0.84**	0.70	**1.50**
SC1S	10.3	*marginata*	315-355	0 - 0.50	**1.82**	**-0.76**	0.70	**1.39**
SC1S	10.3	*marginata*	315-355	0 - 0.50	**1.87**	**-0.89**	0.70	**1.44**
SC1S	10.3	*marginata*	355-425	0 - 0.50	**1.84**	**-0.58**	0.70	**1.41**
SC1K	10.5	*marginata*	< 250	0 - 0.50	**1.76**	**-0.69**	0.70	**1.33**
SC1K	10.5	*marginata*	250-315	0 - 0.50	**1.72**	**-0.55**	0.70	**1.29**
SC1K	10.5	*marginata*	250-315	0 - 0.50	**1.82**	**-0.53**	0.70	**1.39**
SC1K	10.5	*marginata*	315-355	0 - 0.50	**1.73**	**-0.44**	0.70	**1.30**
SC1K	10.5	*marginata*	315-355	0 - 0.50	**1.77**	**-0.38**	0.70	**1.34**
SC1K	10.5	*marginata*	355-425	0 - 0.50	**1.80**	**-0.26**	0.70	**1.37**
SC1K	10.5	*marginata*	355-425	0 - 0.50	**1.62**	**-0.56**	0.70	**1.19**

Appendix 5.1 *(Continued)*

Experiment / Station	Temp. (°C)	Morphotype	Size fraction	Strategy / depth in sediment (cm)	$\delta^{18}O$ (‰ PDB)	$\delta^{13}C$ (‰ PDB)	$\delta^{18}O_w$ (‰ SMOW)	$\delta^{18}O_f$-$\delta^{18}O_w$ (‰ PDB)
OB3G	11.1	*marginata*	200-250	0 - 0.25	**1.73**	**-0.91**	0.70	**1.30**
OB3G	11.1	*marginata*	200-250	0 - 0.25	**1.65**	**-0.90**	0.70	**1.22**
OB3G	11.1	*marginata*	250-315	0 - 0.25	**1.68**	**-0.91**	0.70	**1.25**
OB3G	11.1	*marginata*	250-315	0 - 0.25	**1.62**	**-0.77**	0.70	**1.19**
OB3G	11.1	*marginata*	250-315	0 - 0.25	**1.65**	**-0.84**	0.70	**1.22**
OB3G	11.1	*marginata*	250-315	0 - 0.25	**1.70**	**-0.71**	0.70	**1.27**
OB3G	11.1	*marginata*	250-315	0 - 0.25	**1.74**	**-0.90**	0.70	**1.31**
OB3G	11.1	*marginata*	315-355	0 - 0.25	**1.69**	**-0.78**	0.70	**1.26**
OB3G	11.1	*marginata*	315-355	0 - 0.25	**1.79**	**-0.70**	0.70	**1.36**
OB3G	11.1	*marginata*	315-355	0 - 0.25	**1.74**	**-0.71**	0.70	**1.31**
OB3G	11.1	*marginata*	355-425	0 - 0.25	**1.65**	**-0.42**	0.70	**1.22**
OB3G	11.1	*marginata*	355-425	0 - 0.25	**1.70**	**-0.51**	0.70	**1.27**
OB3G	11.1	*marginata*	355-425	0 - 0.25	**1.65**	**-0.68**	0.70	**1.22**
OB3G	11.1	*marginata*	355-425	0 - 0.25	**1.74**	**-0.28**	0.70	**1.31**
Rhône Prodelta								
Rhône-22	13.3	*marginata*	250-315	3 - 4	**1.53**	**-0.72**	1.45	**0.35**
Rhône-10	13.5	*marginata*	250-315	0 - 0.5	**1.77**	**-0.79**	1.45	**0.59**
Rhône-10	13.5	*marginata*	250-315	0 - 0.5	**1.58**	**-0.90**	1.45	**0.40**
Rhône-12	13.6	*marginata*	250-315	0 - 0.5	**1.82**	**-1.13**	1.45	**0.64**
Rhône-12	13.6	*marginata*	250-315	2 - 5	**1.75**	**-0.93**	1.45	**0.57**
Rhône-12	13.6	*marginata*	250-315	2 - 5	**1.65**	**-1.05**	1.45	**0.47**
Cape Blanc								
Sed-10	4.7	*marginata*	-	6	**2.89**	**-0.59**	0.45	**2.71**
Sed-10	4.7	*marginata*	-	6	**3.00**	**-0.35**	0.45	**2.82**
Sed-11	5.8	*marginata*	-	0	**2.72**	**0.04**	0.45	**2.54**
Sed-11	5.8	*marginata*	-	0	**2.66**	**-0.21**	0.45	**2.48**
Sed-11	5.8	*marginata*	-	0.5	**2.71**	**-0.26**	0.45	**2.53**
Sed-11	5.8	*marginata*	-	1	**2.70**	**-0.27**	0.45	**2.52**
Sed-11	5.8	*marginata*	-	2	**2.73**	**0.00**	0.45	**2.55**
Sed-11	5.8	*marginata*	-	2.5	**2.80**	**-0.13**	0.45	**2.62**
Sed-11	5.8	*marginata*	-	3	**2.77**	**0.01**	0.45	**2.59**
Sed-11	5.8	*marginata*	-	3.5	**2.70**	**-0.34**	0.45	**2.52**
Sed-11	5.8	*marginata*	-	4	**2.81**	**0.00**	0.45	**2.63**
Sed-11	5.8	*marginata*	-	4	**2.76**	**-0.18**	0.45	**2.58**
Sed-11	5.8	*marginata*	-	5	**2.65**	**-0.11**	0.45	**2.47**
Sed-11	5.8	*marginata*	-	6	**3.09**	**-0.04**	0.45	**2.91**
Sed-11	5.8	*marginata*	-	7	**2.58**	**-0.11**	0.45	**2.40**
Sed-15	6.5	*marginata*	-	1	**2.58**	**-0.17**	0.40	**2.45**
Indian Ocean								
MD77-194	6.0	*marginata*	250-315	3.5	**2.92**	**-0.56**	0.10	**3.09**
MD77-194	6.0	*marginata*	250-315	8.5	**2.75**	**-0.26**	0.10	**2.92**
MD77-194	6.0	*marginata*	150-250	8.5	**2.80**	**-0.34**	0.10	**2.97**
MD77-194	6.0	*marginata*	250-315	8.5	**2.69**	**-0.44**	0.10	**2.86**
MD77-194	6.0	*marginata*	250-315	11.5	**2.78**	**-0.47**	0.10	**2.95**
MD77-194	6.0	*marginata*	150-250	15	**2.63**	**-0.28**	0.10	**2.80**

Appendix 5.1 *(Continued)*

Experiment / Station	Temp. (°C)	Morphotype	Size fraction	Strategy / depth in sediment (cm)	$\delta^{18}O$ (‰ PDB)	$\delta^{13}C$ (‰ PDB)	$\delta^{18}O_w$ (‰ SMOW)	$\delta^{18}O_f$-$\delta^{18}O_w$ (‰ PDB)
MD77-194	6.0	*marginata*	250-315	15	**2.87**	**-0.09**	0.10	**3.04**
MD77-194	6.0	*marginata*	150-250	15	**2.53**	**-0.28**	0.10	**2.70**
MD77-194	6.0	*marginata*	250-315	15	**2.64**	**-0.18**	0.10	**2.81**
MD77-194	6.0	*marginata*	250-315	15	**2.53**	**-0.24**	0.10	**2.70**
MD77-194	6.0	*marginata*	250-315	21.5	**2.96**	**0.09**	0.10	**3.13**
MD77-194	6.0	*marginata*	150-250	21.5	**2.65**	**-0.16**	0.10	**2.82**
MD77-194	6.0	*marginata*	250-315	21.5	**2.63**	**-0.19**	0.10	**2.80**
MD77-194	6.0	*marginata*	250-315	25	**2.72**	**-0.27**	0.10	**2.89**
MD77-194	6.0	*marginata*	250-315	25	**2.69**	**-0.50**	0.10	**2.86**
MD77-194	6.0	*marginata*	150-250	35	**2.77**	**-0.36**	0.10	**2.94**
MD77-194	6.0	*marginata*	150-250	35	**2.52**	**-0.46**	0.10	**2.69**
MD77-194	6.0	*marginata*	250-315	35	**2.74**	**-0.25**	0.10	**2.91**
MD77-194	6.0	*marginata*	250-315	35	**2.70**	**-0.38**	0.10	**2.87**
MD77-194	6.0	*marginata*	150-250	39	**2.69**	**-0.12**	0.10	**2.86**
MD77-194	6.0	*marginata*	250-315	39	**3.01**	**-0.15**	0.10	**3.18**
MD77-194	6.0	*marginata*	150-250	39	**2.44**	**-0.28**	0.10	**2.61**
MD77-194	6.0	*marginata*	250-315	39	**2.57**	**-0.06**	0.10	**2.74**
MD77-194	6.0	*marginata*	250-315	45	**2.65**	**-0.16**	0.10	**2.82**
MD77-194	6.0	*marginata*	250-315	45	**2.76**	**-0.22**	0.10	**2.93**
MD77-194	6.0	*marginata*	150-250	48.5	**2.62**	**-0.42**	0.10	**2.79**
MD77-194	6.0	*marginata*	250-315	48.5	**2.61**	**-0.17**	0.10	**2.78**
MD77-194	6.0	*marginata*	150-250	48.5	**2.61**	**-0.37**	0.10	**2.78**
MD77-194	6.0	*marginata*	250-315	48.5	**2.60**	**-0.10**	0.10	**2.77**
MD76-128	3.7	*marginata*	150-250	0	**2.97**	**-0.38**	0.05	**3.19**
MD76-128	3.7	*marginata*	150-250	0	**2.90**	**-0.39**	0.05	**3.12**
MD76-128	3.7	*marginata*	250-315	10	**2.95**	**-0.14**	0.05	**3.17**
MD76-128	3.7	*marginata*	150-250	10	**2.86**	**-0.42**	0.05	**3.08**
MD76-128	3.7	*marginata*	250-315	10	**2.93**	**-0.16**	0.05	**3.15**
MD76-128	3.7	*marginata*	150-250	20	**2.86**	**-0.42**	0.05	**3.08**
MD76-128	3.7	*marginata*	150-250	20	**2.84**	**-0.35**	0.05	**3.06**
MD76-128	3.7	*marginata*	250-315	20	**2.83**	**-0.24**	0.05	**3.05**
MD76-128	3.7	*marginata*	150-250	30	**2.84**	**-0.37**	0.05	**3.06**
MD76-128	3.7	*marginata*	250-315	40	**2.92**	**-0.25**	0.05	**3.14**
MD76-128	3.7	*marginata*	150-250	50	**3.10**	**-0.35**	0.05	**3.32**

SYNTHESE ET PERSPECTIVES

SYNTHESE ET PERSPECTIVES

L'objectif de cette thèse est d'établir une relation quantitative entre la température de l'eau et la composition isotopique en oxygène des coquilles de foraminifères benthiques profonds élevés en culture dans des conditions contrôlées. De nombreuses études expérimentales ont établi des équations de paléotempérature à partir du $\delta^{18}O$ de foraminifères planctoniques, permettant de reconstituer les variations de température de surface des océans. Les foraminifères benthiques sont utilisés afin de reconstituer les températures des eaux de fond. Il est donc nécessaire de réaliser des expériences en laboratoire, permettant de faire varier uniquement la température, afin d'obtenir une calibration précise de ce proxy. Ce travail n'avait, jusqu'à présent, jamais été réalisé avec des foraminifères benthiques profonds.
Des expériences préliminaires avec des assemblages de foraminifères benthiques profonds nous ont conduit à sélectionner l'espèce *Bulimina marginata* (*sensu lato*, cf. Chapitre 2) pour notre travail car elle présentait l'avantage de s'adapter aux conditions de culture en laboratoire. Pour atteindre notre objectif, nous avons mené notre travail en trois phases :

1) Déterminer les conditions optimales de culture permettant d'obtenir la reproduction et la croissance de *B. marginata* en laboratoire ;
2) Mettre en place un protocole expérimental permettant de maintenir nos cultures dans des conditions physico-chimiques stables à long terme, de manière à produire des foraminifères ayant calcifié la totalité de leur coquille dans ces conditions contrôlées ;
3) Etudier l'impact de la température sur la composition isotopique de foraminifères ayant calcifié la totalité de leur coquille dans des conditions expérimentales stables et contrôlées, et estimer l'importance des effets vitaux sur la composition isotopique de ces foraminifères.

Dans un premier temps, nous avons réalisé des cultures en laboratoire de *Bulimina marginata* afin de tester l'effet de différentes conditions de température et d'apports en nourriture sur le comportement de cette espèce en termes de reproduction et de croissance (Chapitre 3).

Des expériences à différentes températures (entre 6 et 14°C) ont montré que les fonctions physiologiques semblent être ralenties lorsque la température est plus basse : la durée

observée avant que les adultes introduits dans l'expérience se reproduisent est plus longue et le taux de croissance de la coquille des juvéniles est plus faible (Figure 1). Quelque soit la température, la même quantité de juvéniles est produite par reproduction mais la quantité totale de juvéniles produits lors de ces expériences présente un maximum aux températures testées intermédiaires (entre 8 et 12°C). Aux températures testées les plus extrêmes, le nombre d'adultes s'étant reproduit était donc certainement plus faible.

Les expériences permettant de tester différents types de nourriture ont montré les effets de la qualité médiocre de la nourriture lyophilisée (algues vertes) comparée à la nourriture fraîche (algues vertes ou diatomées) sur les fonctions de reproduction et de croissance de *B. marginata*. Malgré le fait que les foraminifères aient réussi à se reproduire et à grandir avec les deux types de nourriture, les résultats obtenus étaient meilleurs avec la nourriture fraîche : un délai court avant reproduction, un plus grand nombre de juvéniles produits par reproduction et au total par expérience, et un taux de croissance plus élevé (Figure 1). D'après la littérature, le procédé de lyophilisation a pour conséquence de diminuer le contenu en carbohydrates et en acides gras polyinsaturés. Ces éléments sont essentiels à l'organisme afin qu'il puisse assimiler la nourriture de manière efficace. De plus, nous avons remarqué que les foraminifères élevés en présence de matière organique lyophilisée avaient la particularité de calcifier une coquille très fragile (Figure 1). Au cours du traitement de lyophilisation, les membranes des cellules du phytoplancton sont partiellement détruites, conduisant à une dégradation accélérée de la matière organique par les bactéries. Les modifications de la chimie des carbonates de l'eau de mer résultant de cette minéralisation pourraient provoquer des perturbations au niveau des procédés de calcification chez les foraminifères.

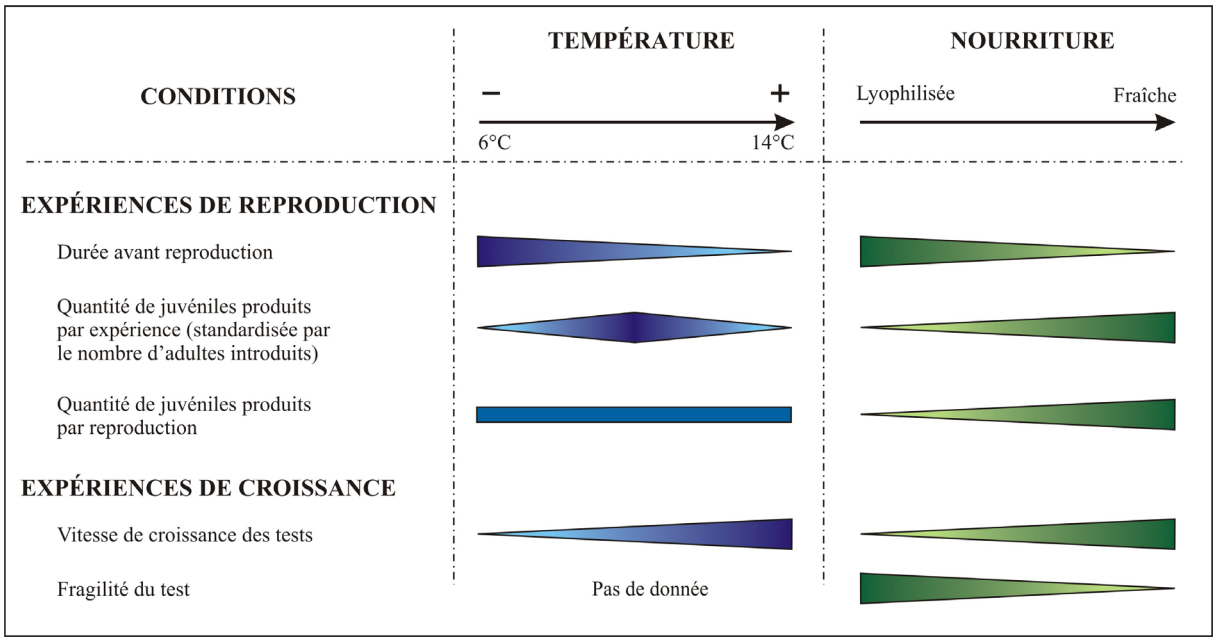


Figure 1 : Influence de différentes conditions de température et de nourriture sur la reproduction et la croissance de Bulimina marginata, *résultats d'expériences en laboratoire (Chapitre 3).*

Cette étude nous a donc permis d'identifier les conditions optimales de culture permettant de produire, dans un temps restreint, des coquilles de foraminifères benthiques profonds, *B. marginata*, en laboratoire. Ces conditions consistent en une source de matière organique fraîche (algues vertes ou diatomées) et des températures autour de 10°C, température du milieu dans lequel les individus de *B. marginata* utilisés dans nos expériences ont été prélevés à l'origine. Cependant, les expériences réalisées par la suite au cours de cette thèse ont prouvé qu'il était possible d'obtenir des reproductions de *B. marginata* entre 4 et 19°C. Les limites de vie de cette espèce sont donc très larges et *B. marginata* peut être considérée comme une espèce eurytherme. Cette caractéristique nous a permis par la suite d'établir nos équations de paléotempérature sur une large gamme de température.

Dans un second temps, nous avons mis au point deux types de systèmes de culture permettant de maintenir des conditions physico-chimiques stables pendant une durée suffisante (entre 40 et 110 jours) pour obtenir la reproduction d'adultes de *B. marginata* et la croissance des juvéniles nés dans ces conditions contrôlées (Chapitre 4).

Notre but était de maintenir les conditions de température et salinité constantes, et par là même la composition isotopique en oxygène de l'eau, mais aussi de maintenir stable le système des carbonates. En effet, tous ces paramètres peuvent avoir une influence sur la composition isotopique des foraminifères. Les suivis de ces paramètres dans deux systèmes, un système fermé et un système en boîte de Pétri, ont démontré qu'il était possible de maintenir des conditions raisonnablement stables.

Pour les expériences en système fermé (CSI et CSII), nous avons obtenu des résultats différents selon la perméabilité des tubes utilisés pour la circulation de l'eau de mer. Le système (CSI) utilisant des tubes en silicone (imperméable aux fluides mais perméable aux gaz) a permis de maintenir les paramètres du système des carbonates constants. En revanche, pour le système (CSII) utilisant des tubes en Tygon® (imperméable aux fluides et aux gaz), nous avons mesuré une acidification de l'eau de mer au cours du temps. L'étanchéité aux gaz du Tygon® aurait bloqué la diffusion vers l'atmosphère du CO_2 produit lors de la dégradation de la matière organique. Ainsi, l'utilisation de tubes en silicone devrait être privilégiée si le but est d'éviter l'évaporation de l'eau de mer tout en gardant le système des carbonates constant (cas de notre étude). Cependant, dans ce cas, l'eau du système est en équilibre avec l'atmosphère et un effort expérimental considérable reste à faire pour aborder la calibration du $\delta^{13}C$ dans la coquille des foraminifères.

Finalement, le système fermé et le système en boîte de Pétri présentent tous deux l'avantage d'être relativement simples à mettre en place et peu couteux. L'intérêt supplémentaire du système en boîte de Pétri est qu'il permet d'observer au fur et à mesure de l'expérience l'évolution des cultures (reproduction, croissance). Cependant il requiert plus de temps de manipulation. Ces systèmes, qui ont été mis au point essentiellement pour l'étude de la température sur la composition isotopique des foraminifères benthiques profonds, pourraient tout à fait être utilisés dans le but de tester l'influence d'autres paramètres (salinité, quantité de matière organique, pH) sur la composition géochimique de foraminifères benthiques moyennant un contrôle de ces paramètres sur de faibles quantités d'eau.

Enfin, nous avons étudié l'influence de la température sur la composition isotopique des coquilles de *B. marginata* obtenues en culture dans les systèmes présentés précédemment (Chapitre 5).

Nous avons réussi à obtenir, dans des conditions contrôlées et stables, des foraminifères ayant calcifié la totalité de leur coquille à des températures comprises entre 4 et 19°C. Grâce au nombre important de foraminifères obtenus dans chacune des expériences, il a été possible de les séparer en fonction de plusieurs classes de taille afin d'étudier l'effet ontogénétique sur la composition isotopique des foraminifères.

Les données isotopiques des coquilles montrent un enrichissement isotopique en oxygène et en carbone avec la croissance. Cet effet ontogénétique est plus important sur la composition en $\delta^{13}C$. Ces observations soulignent l'importance de choisir une fourchette de taille d'individus très restreinte pour faire des mesures isotopiques dans le but de reconstitutions paléocéanographiques.

Par conséquent, nous avons établi les équations de calibration de la composition isotopique en oxygène ($\delta^{18}O_{coquille}$-$\delta^{18}O_{eau}$) des coquilles produites en laboratoire en fonction de la température, pour chacune des classes de taille étudiées (≤ 150 µm, 150-200 µm, 200-250 µm et > 250 µm). Une analyse statistique des données de $\delta^{18}O$ des foraminifères produits dans les différents systèmes de culture a montré que les résultats étaient comparables et pouvaient donc être regroupés, pour une classe de taille donnée. Malheureusement, les données obtenues pour les coquilles de la classe de taille > 250 µm n'étaient pas exploitables à cause du nombre trop restreint de mesures.

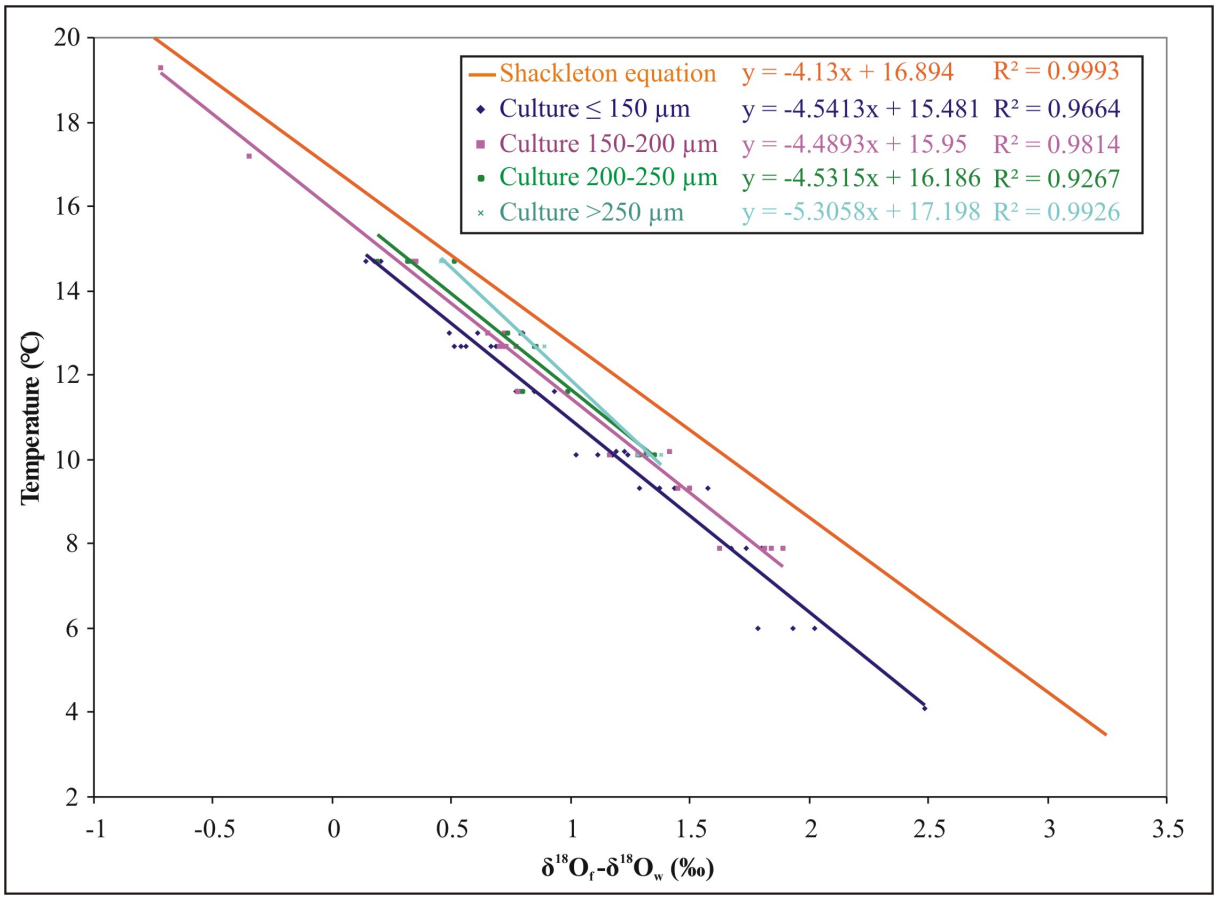


Figure 2 : Calibration de la composition isotopique en oxygène de B. marginata *obtenues en culture ($\delta^{18}O_{coquille}$-$\delta^{18}O_{eau}$) en fonction de la température. L'équation de Shackleton (1974) est aussi indiquée.*

La comparaison entre les droites de calibration ($\delta^{18}O_{coquille}$-$\delta^{18}O_{eau}$ versus température) obtenues à partir des foraminifères de culture et la courbe théorique de la calcite à l'équilibre de Shackleton (1974), adaptée de l'équation de O'Neil *et al*. (1969) pour la calcite inorganique, montre clairement que l'effet de la température est très semblable (Figure 2). Pour les classes de taille ≤ 150 µm, 150-200 µm et 200-250 µm, une augmentation de 1°C de l'eau de mer a pour conséquence une diminution de 0.22‰ du $\delta^{18}O$ des coquilles des foraminifères, ce qui est proche de la valeur de 0.24‰ théorique de Shackleton (1974) pour la calcite à l'équilibre (Figure 2). Parallèlement, plus les foraminifères sont grands, plus leur composition isotopique en oxygène est proche de l'équilibre (Figure 2).

La fraction inférieure à 150 µm est rarement étudiée dans les études paléocéanographiques. Par conséquent, dans le cas de reconstitutions de températures à partir de coquilles de *B. marginata* fossiles, où des individus de large taille ne seraient pas disponibles, nous

recommandons d'analyser des coquilles des fractions 150-200 µm ou 200-250 µm. Du fait de l'effet ontogénétique, les mesures de $\delta^{18}O$ doivent être corrigées respectivement d'un facteur de +0.27‰ et +0.24‰ pour les coquilles de la fraction 150-200 µm et 200-250 µm afin d'atteindre la valeur de la calcite à l'équilibre définie par Shackleton (1974). Alors les équations suivantes peuvent être appliquées :

$$T\,(°C) = 15.95\ (\pm\ 0.14) - 4.49\ (\pm\ 0.13) * (\delta^{18}O_f - \delta^{18}O_w) \qquad R^2 = 0.98$$

Pour la classe de taille 150-200 µm (1)

$$T\,(°C) = 16.23\ (\pm\ 0.35) - 4.61\ (\pm\ 0.37) * (\delta^{18}O_f - \delta^{18}O_w) \qquad R^2 = 0.93$$

Pour la classe de taille 200-250 µm (2)

En conclusion générale, grâce à ce travail de thèse, nous avons réussi à établir un protocole pour produire des coquilles de foraminifères benthiques profonds dans des conditions stables. Les mesures isotopiques réalisées sur les foraminifères obtenus en culture montrent que les résultats sont tout à fait exploitables. Nous avons ainsi pu établir des équations de paléotempérature de manière expérimentale. A notre connaissance, ce sont les premières expériences multi-températures ayant été réalisées à partir de culture de foraminifères benthiques profonds. Ces résultats positifs très encourageants ouvrent la porte pour de multiples études futures de calibration de proxies basées sur la culture. Aussi, de nombreux travaux de recherche pourraient être entrepris en prolongement de ce travail, en paléocéanographie et dans d'autres domaines :

- Les conditions de culture de *B. marginata* étant désormais bien définies, il serait intéressant de réaliser des calibrations d'autres proxies utilisés en paléocéanographie. Un sujet qui intéresse particulièrement la communauté scientifique est l'utilisation de la composition en Mg/Ca des foraminifères, en combinaison avec le $\delta^{18}O$, comme proxy de paléosalinité. A l'aide des systèmes de culture élaborés au cours de notre étude, il serait possible de mettre au point des expériences permettant de mesurer le contenu en Mg/Ca et la composition isotopique de foraminifères ayant calcifié exactement dans les mêmes conditions.
- Il serait évidemment intéressant de réaliser la même étude multi-températures, que celle présentée ici pour l'espèce *B. marginata*, pour des espèces plus courantes en

paléocéanographie telles qu'*Uvigerina*, *Cibicidoides* ou *Globobulimina*. Pour cela, il serait nécessaire de commencer par définir les conditions optimales de vie de ces espèces en laboratoire afin de pouvoir ensuite réaliser des expériences en milieux contrôlés. En effet, ces espèces n'ont encore jamais été utilisées dans les études en laboratoire de calibration de proxies.

- L'étude en laboratoire de l'effet de différents paramètres abiotiques (température, salinité…) ou biotiques (disponibilité et qualité de la matière organique) sur les caractéristiques biologiques (survie, reproduction, croissance) de différentes espèces de foraminifères pourrait présenter un intérêt dans le domaine de la paléoécologie.
- De nos jours, l'acidification des océans, due à l'augmentation de la concentration en dioxyde de carbone atmosphérique, est un réel sujet de préoccupation. En effet, la perturbation de la chimie des carbonates de l'eau de mer pourrait avoir un effet dramatique sur les organismes calcifiant tels que les foraminifères, les coccolithophoridés ou les coraux. La culture en laboratoire de foraminifères benthiques à différentes conditions de pH (ou concentration en CO_3^{2-}) permettrait d'identifier et quantifier les effets d'une acidification de l'eau de mer sur les fonctions vitales de ces organismes et l'impact de telles conditions sur la composition géochimique de leur coquille (isotopes, éléments traces).
- Dans le domaine de l'écotoxicologie, la culture de foraminifères pourrait permettre de mettre en place des bioessais de manière à tester l'impact d'une pollution donnée (e.g. boues de forage, rejet d'eau saturée en sel de stations de désalinisation…) sur la mortalité d'espèces de foraminifères de milieux profonds afin de déterminer son niveau de toxicité.

BIBLIOGRAPHIE

Abu-Zied, R.H., Rohling, E.J., Jorissen, F.J., Fontanier, C., Casford, J.S.L. and Cooke, S., 2008. Benthic foraminiferal response to changes in bottom-water oxygenation and organic carbon flux in the eastern Mediterranean during LGM to Recent times. Marine Micropaleontology 67 (1-2), 46-68.

Albentosa, M., Pérez-Camacho, A., Labarta, U. and Fernandez-Reiriz, J.M., 1997. Evaluation of freeze-dried microalgal diets for the seed culture of Ruditapes decussatus using physiological and biochemical parameters. *Aquaculture* 154, 305-321.

Altenbach, A.V., 1987. The measurement of organic carbon in foraminifera. *Journal of Foraminiferal Research* 17 (2), 106-109.

Altenbach, A.V. and Sarnthein, M., 1989. Productivity Record in Benthic Foraminifera. In: Berger, W.H., Smetacek, V.S., Wefer G. (Editor), Productivity of the Ocean: Present and Past. Wiley, Chichester, pp. 255-269.

Altenbach, A.V., Pflaumann, U., Ralph, S., Thies, A., Timm, S. and Trauth, M., 1999. Scaling and distributional patterns of benthic foraminifera with flux rates of organic carbon. *Journal of Foraminiferal Research* 29, 173-185.

Alve, E. and Bernhard, J.M., 1995. Vertical migratory response of benthic foraminifera to controlled oxygen concentrations in an experimental mesocosm. *Marine Ecology Progress Series* 116, 137-151.

Alve, E. and Goldstein, S.T., 2002. Resting stage in benthic foraminiferal propagules: A key feature for dispersal? Evidence from two shallow-water species. *Journal of Micropalaeontology* 21 (1), 95-96.

Angell, R.W., 1990. Observations on reproduction and juvenile test building in the foraminifer *Trochammina inflata*. *Journal of Foraminiferal Research* 20 (3), 246-247.

Anschutz, P., Sundby, B., Lefrançois, L., Luther III, G.W. and Mucci, A., 2000. Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments. *Geochimica et Cosmochimica Acta* 64, 2751-2763.

Arnold, Z.M., 1954. Culture methods in the study of living foraminifera. *Journal of Paleontology* 28 (4), 404-416.

Babarro, J.M.F., Fernandez-Reiriz, J.M. and Labarta, U., 2001. Influence of preservation techniques and freezing storage time on biochemical composition and spectrum of fatty acids of *Isochrysis galbana* clone T-ISO. *Aquaculture Research* 32, 565-572.

Barmawidjaja, D.M., Jorissen, F.J., Puskaric, S. and van der Zwaan, G.J., 1992. Microhabitat selection by benthic foraminifera in the northern Adriatic Sea. *Journal of Foraminiferal Research* 22 (4), 297-317.

Barnett, P.R.O., Watson, J. and Connely, D., 1984. A multiple corer for taking virtually undisturbed sample from shelf, bathyal and abyssal sediments. *Oceanologica Acta* 7, 399-408.

Barras, C., Geslin, E., Duplessy, J.-C. and Jorissen, F.J., Submitted. Optimisation of laboratory conditions to obtain reproduction and growth of the deep-sea benthic foraminifer *Bulimina marginata*. *Journal of Experimental Marine Biology and Ecology*.

Bartels-Jónsdóttir, H.B., Knudsen, K.L., Abrantes, F., Lebreiro, S. and Eiríksson, J., 2006. Climate variability during the last 2000 years in the Tagus Prodelta, western Iberian Margin: Benthic foraminifera and stable isotopes. *Marine Micropaleontology* 59 (2), 83-103.

Bemis, B.E., Spero, H.J., Bijma, J. and Lea, D.W., 1998. Reevaluation of the oxygen isotopic composition of planktonic foraminifera: Experimental results and revised paleotemperature equations. *Paleoceanography* 13 (2), 150-160.

Bentov, S. and Erez, J., 2005. Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells. *Geology* 33 (11), 841-844.

Berger, W.H., Killingley, J.S. and Vincent, E., 1978. Stable isotopes in deep-sea carbonates: box core ERDC-92 west equatorial Pacific. *Oceanologica Acta* 1, 203-216.

Bergin, F., Kucuksezgin, F., Uluturhan, E., Barut, I.F., Meric, E., Avsar, N. and Nazik, A., 2006. The response of benthic foraminifera and ostracoda to heavy metal pollution in Gulf of Izmir (Eastern Aegean Sea). *Estuarine, Coastal and Shelf Science* 66 (3-4), 368-386.

Bernhard, J.M., 2000. Distinguishing live from dead foraminifera: Methods review and proper applications. *Micropaleontology* 46, 38-46.

Bernhard, J.M., 2003. Potential symbionts in bathyal foraminifera. *Science* 299 (5608), 861.

Bernhard, J.M. and Reimers, C.E., 1991. Benthic foraminiferal population fluctuations related to anoxia: Santa Barbara Basin. *Biogeochemistry* 15 (2), 1577-1585.

Bernhard, J.M. and Alve, E., 1996. Survival, ATP pool, and ultrastructural characterization of benthic foraminiferafrom Drammensfjord (Norway): Response to anoxia. *Marine Micropaleontology* 28 (1), 5-17.

Bernhard, J.M. and Sen Gupta, B.K., 1999. Foraminifera of oxygen-depleted environments. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Press, Dordrecht.

Bernhard, J.M., Blanks, J.K., Hintz, C.J. and Chandler, G.T., 2004. Use of the fluorescent calcite marker calcein to label foraminiferal tests. *Journal of foraminiferal Research* 34, 96-101.

Bernhard, J.M., Ostermann, D.R., Williams, D.S. and Blanks, J.K., 2006. Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions. *Paleoceanography* 21 (4), art. no. PA4210.

Bijma, J., Faber Jr, W.W. and Hemleben, C., 1990. Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. *Journal of Foraminiferal Research* 20 (2), 95-116.

Bijma, J., Hemleben, C., Huber, B.T., Erlenkeuser, H. and Kroon, D., 1998. Experimental determination of the ontogenetic stable isotope variability in two morphotypes of *Globigerinella siphonifera* (d'Orbigny). *Marine Micropaleontology* 35 (3-4), 141-160.

Bouvier-Soumagnac, Y. and Duplessy, J.-C., 1985. Carbon and oxygen isotopic composition of planktonic foraminifera from laboratory culture, plankton tows and recent sediment: implications for the reconstruction of paleoclimatic conditions and of the global carbon cycle. *Journal of Foraminiferal Research* 15 (4), 302-320.

Bouvier-Soumagnac, Y., Duplessy, J.-C. and Bé, A.W.H., 1986. Isotopic composition of a laboratory cultured planktonic foraminifer *O. universa*. Implications for paleoclimatic reconstructions. *Oceanologica Acta* 9 (4), 519-522.

Bradshaw, J.S., 1955. Preliminary laboratory experiments on ecology of foraminiferal populations. *Micropaleontology* 1 (4), 351-358.

Bradshaw, J.S., 1957. Laboratory studies on the rate of growth of the foraminifer, "*Streblus beccarii* (Linné) var. *tepida* (Cushman)". *Journal of Paleontology* 31 (6), 1138-1147.

Bradshaw, J.S., 1961. Laboratory experiments on the ecology of foraminifera. *Contributions from the Cushman Foundation for Foraminiferal Research* 12 (Part 3), 87-106.

Brückner, S. and Mackensen, A., 2008. Organic matter rain rates, oxygen availability, and vital effects from benthic foraminiferal $\delta^{13}C$ in the historic Skagerrak, North Sea. *Marine Micropaleontology* 66 (3-4), 192-207.

Burgess, M.V. and Schnitker, D., 1990. Morphometry of *Bulimina aculeata* Orbigny and *Bulimina marginata* Orbigny. *Journal of Foraminiferal Research* 20 (1), 37-49.

Buscail, R. and Germain, C., 1997. Present-day organic matter sedimentation on the NW Mediterranean margin: Importance of off-shelf export. *Limnology and Oceanography* 42 (2), 217-229.

Buzas, M.A., Culver, S.J. and Jorissen, F.J., 1993. A statistical evaluation of the microhabitats of living (stained) infaunal benthic foraminifera. *Marine Micropaleontology* 20 (3-4), 311-320.

Chandler, G.T., Williams, D.F., Spero, H.J. and Xiaodong, G., 1996. Sediment microhabitat effects on carbon stable isotopic signatures of microcosm-cultured benthic foraminifera. *Limnology and Oceanography* 41 (4), 680-688.

Collins, L.S., 1989. Relationship of environmental gradients to morphologic variation within *Bulimina aculeata* and *Bulimina marginata*, Gulf of Maine area. *Journal of Foraminiferal Research* 19 (3), 222-234.

Copin-Montégut, C. and Bégovic, M., 2002. Distributions of carbonate properties and oxygen along the water column (0-2000 m) in the central part of the NW Mediterranean Sea (Dyfamed site): Influence of winter vertical mixing on air-sea CO_2 and O_2 exchanges. *Deep-Sea Research Part II* 49 (11), 2049-2066.

Cordero Esquivel, B., Voltolina, D. and Correa Sandoval, F., 1993. The biochemical composition of two diatoms after different preservation techniques. *Comparative Biochemistry and Physiology* 105B, 369-373.

Corliss, B.H., 1985. Microhabitats of benthic foraminifera within deep-sea sediments. *Nature* 314, 435-438.

Corliss, B.H., 1991. Morphology and microhabitat preferences of benthic foraminifera from the northwest Atlantic Ocean. *Marine Micropaleontology* 17 (3-4), 195-236.

Corliss, B.H. and Emerson, S., 1990. Distribution of Rose Bengale stained deep-sea benthic foraminifera from the Nova Scotian continental margin and Gulf of Maine. *Deep-Sea Research* 37 (3), 381-400.

Corliss, B.H., McCorkle, D.C. and Higdon, D.M., 2002. A time series study of the carbon isotopic composition of deep-sea benthic foraminifera. *Paleoceanography* 17 (3).

Craig, H., 1965. The measurement of oxygen isotope paleotemperatures. In: Tongiorgi, E. (Editor), Proceedings Spoleto Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures, pp. 3-24.

Cushman, J.A., 1911. A monograph of the foraminifera of the North Pacific Ocean; Part II: Textulariidae. Smithsonian Institution, Washington D.C., 108 pp. pp.

Cushman, J.A. and Parker, F.L., 1947. *Bulimina* and related foraminiferal genera. *US Geological Survey Professional Paper 210-D*, 55–176.

de Nooijer, L.J., Reichart, G.J., Dueñas-Bohórquez, A., Wolthers, M., Ernst, S.R., Mason, P.R.D. and van der Zwaan, G.J., 2007. Copper incorporation in foraminiferal calcite: Results from culturing experiments. *Biogeosciences* 4 (4), 493-504.

de Nooijer, L.J., Duijnstee, I.A.P., Bergman, M.J.N. and van der Zwaan, G.J., 2008. The ecology of benthic foraminifera across the Frisian Front, southern North Sea. *Estuarine, Coastal and Shelf Science* 78 (4), 715-726.

De Rijk, S., Troelstra, S.R. and Rohling, E.J., 1999. Benthic formainiferal distribution in the Mediterranean Sea. *Journal of Foraminiferal Research* 29 (2), 93-103.

De Rijk, S., Jorissen, F.J., Rohling, E.J. and Troelstra, S.R., 2000. Organic flux control on bathymetric zonation of Mediterranean benthic foraminifera. *Marine Micropaleontology* 40 (3), 151-166.

de Stigter, H.C., 1996. Recent and fossil foraminifera in the Adriatic Sea: distribution patterns in relation to organic carbon flux and oxygen concentration at the seabed, Geologica Ultraiectina

Debenay, J.-P. and Redois, F., 1997. Distribution of the twenty seven dominant species of shelf benthic foraminifers on the continental shelf, north of Dakar (Senegal). *Marine Micropaleontology* 29 (3-4), 237-255.

Debenay, J.-P., Guillou, J.-J., Geslin, E. and Lesourd, M., 2000. Crystallization of calcite in foraminiferal tests. *Micropaleontology* 46, 87-94.

Delaygue, G., Bard, E., Rollion, C., Jouzel, J., Stievenard, M., Duplessy, J.-C. and Ganssen, G., 2001. Oxygen isotope/salinity relationship in the northern Indian Ocean. *Journal of Geophysical Research* 106 (C3), 4565-4574.

Di Leonardo, R., Bellanca, A., Capotondi, L., Cundy, A. and Neri, R., 2007. Possible impacts of Hg and PAH contamination on benthic foraminiferal assemblages: An example from the Sicilian coast, central Mediterranean. *Science of the Total Environment* 388 (1-3), 168-183.

Dickson, A.G.J.C.T., 1990. Standard potential of the reaction – $AgCl_{(s)}$ + $1/2H2_{(g)}$ = $Ag_{(s)}$ +$HCl_{(aq)}$ and the standard acidity constant of the ion HSO_4^- in synthetic sea-water from 273.15 K to 318.15 K. *The Journal of Chemical Thermodynamics* 22, 113-127.

Divakar Naidu, P., Ramesh Kumar, M.R. and Ramesh Babu, V., 1999. Time and space variations of monsoonal upwelling along the west and east coasts of India. *Continental Shelf Research* 19 (4), 559-572.

Donnici, S. and Barbero, R., 2002. The benthic foraminiferal communities of the northern Adriatic continental shelf. *Marine Micropaleontology* 44 (3-4), 93-123.

d'Orbigny, A., 1826. Tableau méthodique de la classe des Céphalopodes. *Annales des Sciences Naturelles* 1 (7), 96-317.

Dunbar, R.B. and Wefer, G., 1984. Stable isotope fractionation in benthic foraminifera from the Peruvian continental margin. *Marine Geology* 59, 215-225.

Duplessy, J.-C., Lalou, C. and Vinot, A.C., 1970. Differential Isotopic Fractionation in Benthic Foraminifera and Paleotemperatures Reassessed. *Science* 168, 250-251.

Duplessy, J.-C. and Fieux, M., 1972. Etude isotopique des apports d'eau douce en Méditerranée occidentale. *Rapport de la Commission Internationale d'étude de la Mer Méditerranée* 21, 197-201.

Eberwein, A. and Mackensen, A., 2006. Regional primary productivity differences off Morocco (NW-Africa) recorded by modern benthic foraminifera and their stable carbon isotopic composition. *Deep Sea Research I* 53 (8), 1379-1405.

Eggins, S., De Deckker, P. and Marshall, J., 2003. Mg/Ca variation in planktonic foraminifera tests: Implications for reconstructing -palaeo-seawater temperature and habitat migration. *Earth and Planetary Science Letters* 212 (3-4), 291-306.

Eichler, P.P.B., Sen Gupta, B.K., Eichler, B.B., Braga, E.S. and Campos, E.J., 2008. Benthic foraminiferal assemblages of the South Brazil: Relationship to water masses and nutrient distributions. *Continental Shelf Research* 28 (13), 1674-1686.

Elderfield, H., Vautravers, M. and Cooper, M., 2002. The relationship between size and Mg/Ca, Sr/Ca, $d^{18}O$, and $d^{13}C$ of species of planktonik foraminifera. *Geochemistry Geophysics Geosystems* 3 (8).

Emiliani, C., 1955. Pleistocene temperatures. *Journal of Geology* 63 (538-578).

Enright, C.T., Newkirk, G.F., Craigie, J.S. and Castell, J.D., 1986. Growth of juvenile Ostrea edulis L. fed *Chaetoceros gracilis* Schuett of varied chemical composition. *Journal of Experimental Marine Biology and Ecology* 96 (1), 15-26.

Epstein, S.R., Buchsbaum, R., Lowenstem, H.A. and Urey, H.C., 1953. Revised carbonate-water isotopic temperature scale. *Geol. Soc. Am. Bull.* 64, 1315-1326.

Erez, J., 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. *Nature* 273, 199-202

Erez, J. and Luz, B., 1983. Experimental paleotemperature equation from planktonic forminifera. *Geochimica et Cosmochimica Acta* 47 (6), 1025-1031.

Ernst, S., Duijnstee, I. and van der Zwaan, B., 2002. The dynamics of the benthic foraminiferal microhabitat: Recovery after experimental disturbance. *Marine Micropaleontology* 46 (3-4), 343-361.

Ernst, S. and van der Zwaan, B., 2004. Effects of experimentally induced raised levels of organic flux and oxygen depletion on a continental slope benthic foraminiferal community. *Deep-Sea Research Part I* 51 (11), 1709-1739.

Ernst, S., Bours, R., Duijnstee, I. and van der Zwaan, B.D., 2005. Experimental effects of an organic matter pulse and oxygen depletion on a benthic foraminiferal shelf community. *Journal of Foraminiferal Research* 35 (3), 177-197.

Ernst, S., Morvan, J., Geslin, E., Le Bihan, A. and Jorissen, F.J., 2006. Benthic foraminiferal response to experimentally induced Erika oil pollution. *Marine Micropaleontology* 61 (1-3), 76-93.

Fontanier, C., Jorissen, F.J., Licari, L., Alexandre, A., Anschutz, P. and Carbonel, P., 2002. Live benthic foraminiferal faunas from the Bay of Biscay: faunal density, composition, and microhabitats. *Deep-Sea Research I* 49, 751-785.

Fontanier, C., Mackensen, A., Jorissen, F.J., Anschutz, P., Licari, L. and Griveaud, C., 2006. Stable oxygen and carbon isotopes of live benthic foraminifera from the Bay of Biscay : Microhabitat impact and seasonal variability. *Marine Micropaleontology* 58 (3), 159-183.

Fornasini, C., 1902. Sinossi metodica dei foraminiferi sin qui rinvenuti nella sabbia del Lido di Rimini. *Reale Accademia delle Scienze dell'Istituto di Bologna, Memorie di Scienze Naturali* (ser. 5), vol. 9.

Frew, R.D., Dennis, P.F., Heywood, K.J., Meredith, M.P. and Boswell, S.M., 2000. The oxygen isotope composition of water masses in the northern North Atlantic. *Deep-Sea Research I* 47, 2265-2286.

Frezza, V. and Carboni, M.G., in press. Distribution of recent foraminiferal assemblages near the Ombrone River mouth (Northern Tyrrhenian Sea, Italy). *Revue de Micropaléontologie*.

Froelich, P.N., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochimica et Cosmochimica Acta* 43 (7), 1075-1090.

Fütterer, D., 1983. The modern upwelling record off Northwest Africa. In: Thiede, J. and Suess, E. (Editors), Coastal Upwelling. Its Sediment Record. Part B. Sedimentary Records of Ancient Coastal Upwellings IV, Marine Sciences, vol 10B. Nato Conference Series, pp. p. 105-121.

Gadel, F., Puigbó, A., Alcañiz, J.M., Charrière, B. and Serve, L., 1990. Tracers and constituents indicating the nature of organic fluxes, their origin and the effect of environmental conditions. *Continental Shelf Research* 10 (9-11), 1039-1062.

Gerlach, S.A., Hahn, A.E. and Schrage, M., 1985. Size spectra of benthic biomass and metabolism. *Marine Ecology Progress Series* 26, 161-173.

Geslin, E., Heinz, P., Jorissen, F. and Hemleben, C., 2004. Migratory responses of deep-sea benthic foraminifera to variable oxygen conditions: Laboratory investigations. *Marine Micropaleontology* 53 (3-4), 227-243.

Goldstein, S.T., 1999. Foraminifera: A Biological Overview. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Publishers, pp. 37-55.

Goldstein, S.T. and Moodley, L., 1993. Gametogenesis and the life cycle of the foraminifer *Ammonia beccarii* (Linne) forma *tepida* (Cushman). *Journal of Foraminiferal Research* 23 (4), 213-220.

Goldstein, S.T. and Corliss, B.H., 1994. Deposit feeding selected deep-sea and shallow-water benthic foraminifera. *Deep-Sea Research I* 41 (2), 229-241.

Gooday, A.J., 1986. Meiofaunal foraminiferans from the bathyal Porcupine Seabight (northeast Atlantic): size structure, standing stock, taxonomy composition, species diversity and vertical distribution in the sediment. *Deep Sea Research* 33 (10), 1345-1373.

Gooday, A.J., Levin, L.A., Linke, P. and Heeger, T., 1992. The role of benthic foraminifera in deep-sea food webs and carbon cycling. In: Rowe, G.T.a.P., V. (Editor), Deep-sea Food Chains and the Global Carbon Cycle. Kluwer Academic publishers, The Netherlands, pp. 63-91.

Gooday, A.J., Bernhard, J.M., Levin, L.A. and Suhr, S.B., 2000. Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen-deficient settings: Taxonomic composition, diversity, and relation to metazoan faunas. *Deep-Sea Research Part II* 47 (1-2), 25-54

Gran, G., 1952. Determination of the equivalence point in potentiometric titrations. Part II. *The Analyst* 77 (920), 661-670.

Griveaud, C., 2007. Influence des conditions écologiques sur la composition isotopique ($\delta^{13}C$, $\delta^{18}O$) de tests de foraminifères benthiques actuels, University of Angers, Angers (PhD Thesis)

Gross, O., 2000. Influence of temperature, oxygen and food availability on the migrational activity of bathyal benthic foraminifera: Evidence by microcosm experiments. *Hydrobiologia* 426 (1-3), 123-137.

Grossman, E.L., 1987. Stable isotopes in modern benthic foraminifera: a study of vital effect. *Journal of Foraminiferal Research* 17 (1), 48-61.

Guillard, R.R.L. and Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. *Canadian Journal of Microbiology* 8, 229-239.

Gulati, R.D. and Demott, W.R., 1997. The role of food quality for zooplankton: Remarks on the state -of-the-art, perspectives and priorities. *Freshwater Biology* 38 (3), 753-768.

Haake, B., Ittekkot, V., Rixen, T., Ramaswamy, V., Nair, R.R. and Curry, W.B., 1993. Seasonality and interannual variability of particle fluxes to the deep Arabian Sea. *Deep-Sea Research I* 40, 1323–1344.

Hall, P.O.J. and Aller, R.C., 1992. Rapid, small-volume, flow injection analysis for ΣCO_2 and NH_4^+ in marine and freshwaters. *Limnology and Oceanography* 37 (5), 1113-1119.

Hallock, P., Cottey, T.L., Forward, L.B. and Halas, J., 1986. Population biology and sediment production of *Archaias angulatus* (Foraminiferida) in Largo Sound, Florida. *Journal of Foraminiferal Research* 16 (1), 1-18.

Hannah, F., Rogerson, A. and Laybourn-Parry, J., 1994. Respiration rates and biovolumes of common benthic foraminifera (protozoa). *Journal of the Marine Biological Association of the UK* 74, 301-312.

Havach, S.M., Chandler, G.T., Wilson-Finell, A. and Shaw, T.J., 2001. Experimental determination of trace element partition coefficients in cultured benthic foraminifera. *Geochimica et Cosmochimica Acta* 65 (8), 1277-1283.

Hayward, B.W., Grenfell, H.R., Reid, C.M. and Hayward, K.A., 1999. Recent New Zealand shallow-waterbenthic foraminifera: Taxonomy, ecologic distribution, niogeography, and use in paleoenvironmental assessment. Graphic Press & Packaging Ltd, Levin.

Hayward, B.W., Grenfell, H.R., Sabaa, A.T. and Neil, H.L., 2007. Factors influencing the distribution of Subantarctic deep-sea benthic foraminifera, Campbell and Bounty Plateaux, New Zealand. *Marine Micropaleontology* 62 (3), 141-166.

Heinz, P., Kitazato, H., Schmiedl, G. and Hemleben, C., 2001. Response of deep-sea benthic foraminifera from the Mediterranean Sea to simulated phytoplankton pulses under laboratory conditions. *Journal of Foraminiferal Research* 31 (3), 210-227.

Heinz, P., Hemleben, C. and Kitazato, H., 2002. Time-response of cultured deep-sea benthic foraminifera to different algal diets. *Deep-Sea Research I* 49 (3), 517-537.

Heinz, P. and Hemleben, C., 2003. Regional and seasonal variations of recent benthic deep-sea foraminifera in the Arabian Sea. *Deep-Sea Research Part I* 50 (3), 435-447.

Heinz, P., Geslin, E. and Hemleben, C., 2005. Laboratory observations of benthic foraminiferal cysts. *Marine Biology Research* 1, 149-159.

Hemleben, C. and Kitazato, H., 1995. Deep-sea foraminifera under long time observation in the laboratory. *Deep Sea Research I* 42 (6), 827-832.

Herguera, J.C. and Berger, W.H., 1991. Paleoproductivity from benthic foraminifera abundance; glacial and postglacial change in the west-equatorial Pacific. *Abstracts with Program Geological Society of America* 23 (5), 107.

Hess, S., Jorissen, F.J., Venet, V. and Abu-Zied, R., 2005. Benthic foraminiferal recovery after recent turbidite deposition in Cap Breton canyon, Bay of Biscay. *Journal of Foraminiferal Research* 35 (2), 114-129.

Hess, S. and Jorissen, F., in press. Distribution patterns of living benthic foraminifera from Cap Breton Canyon, Bay of Biscay: faunal response to sediment instability. *Deep Sea Research I.*

Hintz, C.J., Chandler, G.T., Bernhard, J.M., McCorkle, D.C., Havach, S.M., Blanks, J.K. and Shaw, T.J., 2004. A physicochemically constrained seawater culturing system for production of benthic foraminifera. *Limnology and Oceanography: Methods* 2, 160-170.

Hintz, C.J., Shaw, T.J., Chandler, G.T., Bernhard, J.M., McCorkle, D.C. and Blanks, J.K., 2006a. Trace/minor element:calcium ratios in cultured benthic foraminifera. Part I: Inter-species and inter-individual variability. *Geochimica et Cosmochimica Acta* 70 (8), 1952-1963.

Hintz, C.J., Shaw, T.J., Bernhard, J.M., Chandler, G.T., McCorkle, D.C. and Blanks, J.K., 2006b. Trace/minor element:calcium ratios in cultured benthic foraminifera. Part II: Ontogenetic variation. *Geochimica et Cosmochimica Acta* 70 (8), 1964-1976.

Hoeglund, H., 1947. Foraminifera in the Gullmar Fjord and the Skagerack. *Zoologiska Bidrag Fran Uppsala* 26, 1-328.

Horibe, Y. and Oba, T., 1972. Temperature scales of aragonite-water and calcite-water systems. *Fossils* 23/24, 69-79.

Hottinger, L., Halicz, E. and Reiss, Z., 1993. Recent foraminiferida from the Gulf of Aqaba, Red Sea.

Hut, G., 1987. Consultants group meeting on stable isotope reference samples for geochemical and hydrological investigations, Vienna.

Hyacinthe, C., Anschutz, P., Carbonel, P., Jouanneau, J.-M. and Jorissen, F.J., 2001. Early diagenetic processes in the muddy sediments of the Bay of Biscay. *Marine Geology* 177, 111-128.

Igarashi, A., Numanami, H., Tsuchiya, Y. and Fukuchi, M., 2001. Bathymetric distribution of fossil foraminifera within marine sediment cores from the eastern part of Lützow-Holm Bay, East Antarctica, and its paleoceanographic implications. *Marine Micropaleontology* 42 (3-4), 125-162.

Jannink, N.T., Zachariasse, W.J. and van der Zwaan, G.J., 1998. Living (Rose Bengal stained) benthic foraminifera from the Pakistan continental margin (northern Arabian Sea). *Deep-Sea Research I* 45, 1483-1513.

Jian, Z., Wang, L., Kienast, M., Sarnthein, M., Kuhnt, W., Lin, H. and Wang, P., 1999. Benthic foraminiferal paleoceanography of the South China Sea over the last 40,000 years. *Marine Geology* 156 (1-4), 159-186.

Jones, T.R., 1875. In: Wright, J.A. (Editor), List of the Cretaceous Microzoa of the North of Ireland. Proceedings of the Belfast Naturalists' Field Club pp. 73–99.

Jorissen, F., 1988. Benthic foraminifera from the Adriatic Sea; Principles of phenotypic variation. *Utrecht Micropaleontological Bulletins* 37, 174 pp.

Jorissen, F.J., 1999a. Benthic foraminiferal microhabitats. In: Sen Gupta, B.K. (Editor), Foraminifera. Kluwer Academic Publishers, Dordrecht, pp. 161-179.

Jorissen, F.J., 1999b. Benthic foraminiferal successions across Late Quaternary Mediterranean Sapropels. *Marine Geology* 153, 91–103.

Jorissen, F.J., de Stigter, H.C. and Widmark, J.G.V., 1995. A conceptual model explaining benthic foraminiferal microhabitats. *Marine Micropaleontology* 22, 3-15.

Jorissen, F.J., Wittling, I., Peypouquet, J.P., Rabouille, C. and Relexans, J.C., 1998. Live benthic foraminiferal faunas off Cap Blanc, NW Africa: community structure and microhabitats. *Deep-Sea Research I* 45, 2157-2188.

Jorissen, F.J., Fontanier, C. and Thomas, E., 2007. Chapter Seven Paleoceanographical Proxies Based on Deep-Sea Benthic Foraminiferal Assemblage Characteristics. In: Hillaire-Marcel, C. and De Vernal, A. (Editors), Paleoceanography of the Late Cenozoic. Developments in Marine Geology. Elsevier, pp. 263-325.

Kawagata, S., Hayward, B.W., Grenfell, H.R. and Sabaa, A., 2005. Mid-Pleistocene extinction of deep-sea foraminifera in the North Atlantic Gateway (ODP sites 980 and 982). *Palaeogeography, Palaeoclimatology, Palaeoecology* 221 (3-4), 267-291.

Kim, S.-T. and O'Neil, J.R., 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochimica et Cosmochimica Acta* 61 (16), 3461-3475.

Kitazato, H., 1989. Vertical distribution of benthic foraminifera within sediments (Preliminary Report). *Benthos Results (Bulletin Japonese Association of Benthology)* 35/36, 41-51.

Kitazato, H., Tazume, M. and Tsuchiya, M., 2003. Phylogenetic relationship among species of genus *Bulimina* (Foraminifera) inferred from ribosomal DNA sequences. *Frontier Research on Earth Evolution* 1, 233-238.

Koho, K.A., Kouwenhoven, T.J., de Stigter, H.C. and van der Zwaan, G.J., 2007. Benthic foraminifera in the Nazaré Canyon, Portuguese continental margin: Sedimentary environments and disturbance. *Marine Micropaleontology* 66 (1), 27-51.

Kurbjeweit, F., Hemleben, C., Schmiedl, G., Schiebel, R., Pfannkuche, O., Wallmann, K. and Schäfer, P., 2000. Distribution, biomass and diversity of benthic foraminifera in relation to sediment geochemistry in the Arabian Sea. *Deep-Sea Research II* 47 (14), 2913–2955.

Laborde, P., Urrutia, J. and Valencia, V., 1999. Seasonal variability of primary production in the Cap-Ferret Canyon area (Bay of Biscay) during the ECOFER cruises. *Deep-Sea Research II* 46, 2057-2079.

Laing, I., Child, A.R. and Janke, A., 1990. Nutritional value of dried algae diets for larvae of Manila clam (*Tapes philippinarum*). *Journal of the Marine Biological Association of the UK* 70, 1-12.

Langezaal, A.M., Jorissen, F.J., Braun, B., Chaillou, G., Fontanier, C., Anschutz, P. and van der Zwaan, G.J., 2006. The influence of seasonal processes on geochemical profiles and foraminiferal assemblages on the outer shelf of the Bay of Biscay. *Continental Shelf Research* 26 (15), 1730-1755.

Le Cadre, V. and Debenay, J.-P., 2006. Morphological and cytological responses of *Ammonia* (foraminifera) to copper contamination: Implication for the use of foraminifera as bioindicators of pollution. *Environmental Pollution* 143 (2), 304-317.

Lea, D.W., 1999. Trace elements in foraminiferal calcite. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Publishers, Dordrecht, pp. 259–277.

Lee, J.J. and Anderson, O.R. (Editors), 1991. Biology of Foraminifera. Academic Press, 368 pp.

Lee, J.J., Faber, W.W., Anderson, O.R. and Pawlowski, J., 1991. Life cycles of foraminifera. In: Lee, J.J. and Anderson, J. (Editors), Biology of Foraminifera. Academic Press, London, pp. 285-334.

Lee, J.J., Morales, J., Symons, A. and Hallock, P., 1995. Diatom symbionts in larger foraminifera from Caribbean hosts. *Marine Micropaleontology* 26 (1-4), 99-105.

Levin, L.A. and Gage, J.D., 1998. Relationships between oxygen, organic matter and the diversity of bathyal macrofauna. *Deep-Sea Research Part II* 45 (1-3), 129-163.

Lewis, E. and Wallace, D.W.R., 1998. Program Developed for CO2 System Calculations, ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U. S. Department of Energy, Oak Ridge, Tennessee.

Licari, L.N., Schumacher, S., Wenzhöfer, F., Zabel, M. and Mackensen, A., 2003. Communities and microhabitats of living benthic foraminifera from the tropical east Atlantic: impact of different productivity regimes. *Journal of Foraminiferal Research* 33, 10-31.

Lin, L.I., 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45 (1), 255-268.

Loeblich, A.R. and Tappan, H., 1964. Sarcodina, chiefly thecamoebians and Foraminiferida. Geological Society of America, New York, 510+390 pp.

Loeblich, A.R. and Tappan, H., 1988. Foraminiferal genera and their classification. Van Nostrand Reinhold, New-York, 970 pp.

Lutze, G.F. and Coulbourn, W.T., 1984. Recent benthic foraminifera from the continental margin of Northwest Africa: community structure and distribution. *Marine Micropaleontology* 8 (5), 361-401.

Lynch-Stieglitz, J., Curry, W.B. and Slowey, N., 1999. A geostrophic transport estimate for the Florida Current from the oxygen isotope composition of benthic foraminifera. *Paleoceanography* 14 (3), 360-373.

Mackensen, A., Hubberten, H.-W., Bickert, T., Fischer, G. and Fütterer, D.K., 1993. The $\delta^{13}C$ in benthic foraminiferal tests of *Fontbotia wuellerstorfi* (Schwager) relative to the $\delta^{13}C$ of dissolved inorganic carbon in southern ocean deep water: implications for glacial ocean circulation models. *Paleoceanography* 8 (5), 587-610.

Mackensen, A., Schmiedl, G., Harloff, J. and Giese, M., 1995. Deep-sea foraminifera in the South Atlantic Ocean: Ecology and assemblage generation. *Micropaleontology* 41 (4), 342-358.

Mackensen, A. and Bickert, T., 1999. Stable carbon isotopes in benthic foraminifera: proxies for deep and bottom water circulation and new production. In: Fischer, G. and Wefer, G. (Editors), Use of proxies in paleoceanography: Examples from the South Atlantic. Springer Verlag, Berlin, pp. 229–254.

Mackensen, A., Schumacher, S., Radke, J. and Schmidt, D.N., 2000. Microhabitat preferences and stable carbon isotopes of endobenthic foraminifera: clue to quantitative reconstruction of oceanic new production? *Marine micropaleontology* 40 (3), 233-258.

Mackensen, A. and Licari, L., 2004. Carbon isotopes of live benthic foraminifera from the South Atlantic Ocean: sensitivity to bottom water carbonate saturation state and organic matter rain rates. In: G. Wefer, S.M.a.V.R. (Editor), The South Atlantic in the Late Quaternary : Reconstruction of Material Budget and Current Systems 261. Springer-Verlag, New-York, pp. 623-644.

Marsaleix, P., 1993. Modelisation Tridimensionnelle de la Circulation Océanique dans le Golfe du Lion, Toulouse (PhD thesis)

Martins, M.V.A. and Gomes, V.C.R.D., 2004. Foraminíferos da margem Continental NW Ibérica: Sistemática, Ecologia e Distribuição. Tiragem.

McConnaughey, T., 1989a. ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. *Geochimica et Cosmochimica Acta* 53 (1), 151-162.

McConnaughey, T., 1989b. ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: II. *In vitro* simulation of kinetic isotope effects. *Geochimica et Cosmochimica Acta* 53 (1), 163-171.

McCorkle, D.C., Emerson, S.R. and Quay, P.D., 1985. Stable isotopes in marine porewaters. *Earth and Planetary Science Letters* 74, 13-26.

McCorkle, D.C., Keigwin, L.D., Corliss, B. and Emerson, S.R., 1990. The influence of microhabitats on the carbon isotopic composition of deep-sea benthic foraminifera. *Paleoceanography* 5 (2), 161-185.

McCorkle, D.C., Corliss, B.H. and Farnham, C.A., 1997. Vertical distributions and stable isotopic compositions of live (stained) benthic foraminifera from the North Carolina and California continental margins. *Deep-Sea Research Part I* 44 (6), 983-1024.

McCorkle, D.C., Bernhard, J.M., Hintz, C.J., Blanks, J.K., Chandler, G.T. and Shaw, T.J., 2004. The Carbon and Oxygen Stable Isotopic Composition of Cultured Benthic Foraminifera, 8th International Conference on Paleoceanography, Biarritz, France.

McCrea, J.M., 1950. On the isotopic chemistry of carbonates and a paleotemperature scale. *The Journal of Chemical Physics* 18 (6), 849-857.

McCullough, D., 1999. A Review and Synthesis of Effects of Alterations to the Water Temperature Regime on Freshwater Life Stages of Salmonids, with Special Reference to Chinook Salmon, Columbia Intertribal Fisheries Commission, Portland, OR, pp. 279.

Mendes, I., Gonzalez, R., Dias, J.M.A., Lobo, F. and Martins, V., 2004. Factors influencing recent benthic foraminifera distribution on the Guadiana shelf (Southwestern Iberia). *Marine Micropaleontology* 51, 171– 192.

Millot, C., 1990. The Gulf of Lions' hydrodynamics. *Continental Shelf Research* 10, 885-894.

Mojtahid, M., 2007. Les foraminifères benthiques : bio-indicateurs d'eutrophisation naturelle et anthropique en milieu marin franc, Angers (PhD Thesis)

Mojtahid, M., Jorissen, F., Durrieu, J., Galgani, F., Howa, H., Redois, F. and Camps, R., 2006. Benthic foraminifera as bio-indicators of drill cutting disposal in tropical east Atlantic outer shelf environments. *Marine Micropaleontology* 61 (1-3), 58-75.

Mojtahid, M., Jorissen, F. and Pearson, T.H., 2008. Comparison of benthic foraminiferal and macrofaunal responses to organic pollution in the Firth of Clyde (Scotland). *Marine Pollution Bulletin* 56 (1), 42-76.

Mojtahid, M., Jorissen, F., Lansard, B., Fontanier, C., Bombled, B. and Rabouille, C., Submitted. Spatial distribution of benthic foraminifera in the Rhône prodelta: faunal response to organic matter focussing. *Continental Shelf Research*.

Monaco, A., Biscaye, A., Soyer, J., Pocklington, R. and Heussner, S., 1990. Particle fluxes and ecosystem response on a continental margin: the 1985–1988 Mediterranean ECOMARGE experiment. *Continental Shelf Research* 10 (9-11), 809-839.

Morigi, C., Jorissen, F.J., Gervais, A., Guichard, S. and Borsetti, A.M., 2001. Benthic foraminiferal faunas in surface sediments off NW Africa: Relationship with the organic flux to the ocean floor. *Journal of Foraminiferal Research* 31, 350-368.

Murgese, D.S. and De Deckker, P., 2007. The Late Quaternary evolution of water masses in the eastern Indian Ocean between Australia and Indonesia, based on benthic foraminifera faunal and carbon isotopes analyses. *Paleogeography, Paleoclimatology, Paleoecology* 247 (3-4), 382-401.

Murray, J.W., 1963. Ecological experiments on foraminiferida. *Journal of the Marine Biological Association of the UK* 43, 621-642.

Murray, J.W., 1983. Population dynamics of benthic foraminifera: Results from the Exe estuary, England. *Journal of Foraminiferal Research* 13 (1), 1-12.

Murray, J.W., 1991. Ecology and paleoecology of benthic foraminifera. Longman Scientific & Technical, UK, 397 pp.

Murray, J.W., 2006. Ecology and applications of benthic foraminifera. Cambridge University Press, Cambridge, 426 pp.

Myers, E.H., 1935. Culture Methods for the Marine Foraminifera of the Littoral Zone. *Transactions of the American Microscopical Society* 54, 264-267.

Myers, E.H., 1943. Life activities of foraminifera in relation to marine ecology. *Proceedings of the American Philosophical Society* 86, 439-458.

Nigam, R. and Caron, D.A., 2000. Does temperature affect dimorphic reproduction in benthic foraminifera? A culture experiment on *Rosalina leei*. *Current Science* 79 (8), 1105-1106.

Nomaki, H., Heinz, P., Hemleben, C. and Kitazato, H., 2005a. Behavior and response of deep-sea benthic foraminifera to freshly supplied organic matter: A laboratory feeding experiment in microcosm environments. *Journal of foraminiferal Research* 35 (2), 103-113.

Nomaki, H., Heinz, P., Nakatsuka, T., Shimanaga, M. and Kitazato, H., 2005b. Species-specific ingestion of organic carbon by deep-sea benthic foraminifera and meiobenthos: *In situ* tracer experiments. *Limnology and Oceanography* 50 (1), 134-146.

Nomaki, H., Heinz, P., Nakatsuka, T., Shimanaga, M., Ohkouchi, N., Ogawa, N.O., Kogure, K., Ikemoto, E. and Kitazato, H., 2006. Different ingestion patterns of ^{13}C-labeled bacteria and algae by deep-sea benthic foraminifera. *Marine Ecology Progress Series* 310 (95-108).

Nürnberg, D., Bijma, J. and Hemleben, C., 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochimica et Cosmochimica Acta* 60 (5), 803-814.

Ogawa, N. and Tauzin, P., 1973. Contribution à l'étude hydrologique et géochimique du Golfe de Cap-Breton. *Bulletin de l'Institut Géologique du Bassin d'Aquitaine, Bordeaux* 14, 19-46.

Ohga, T. and Kitazato, H., 1997. Seasonal changes in bathyal foraminiferal populations in response to the flux of organic matter (Sagami Bay, Japan). *Terra Nova* 9 (1), 33-37.

Olivieri, R., 1996. Cronologia ed interpretazione paleoambientale del pleistocene inferiore del pozzo C.N.R. Venezia 1. , University of Padua

O'Neil, J.R., Clayton, R.N. and Mayeda, T.K., 1969. Oxygen isotope fractionation in divalent metal carbonates. *The Journal of Chemical Physics* 51 (12), 5547-5558.

Oppo, D.W. and Fairbanks, R.G., 1989. Carbon isotopic comparison of tropical surface water during the past 22,000 year. *Paleoceanography* 4, 333-351.

Panieri, G. and Sen Gupta, B.K., 2008. Benthic Foraminifera of the Blake Ridge hydrate mound, Western North Atlantic Ocean. *Marine Micropaleontology* 66 (2), 91-102.

Parker, W.K., Jones, T.R. and Brady, H.B., 1871. On the nomenclature of the foraminifera in the Annales des Sciences Naturelles, tome VII, the species founded upon the figures in Soldani's Testaceographia ae zoophytographia. *Annals and magazine of Natural History* 4, 145-179, 283-266.

Pascual, A., Rodriguez-Lazaro, J., Martín-Rubio, M., Jouanneau, J.-M. and Weber, O., In press. A survey of the benthic microfauna (foraminifera, Ostracoda) on the Basque shelf, southern Bay of Biscay. *Journal of Marine Systems*.

Pawlowski, J., 2000. Introduction to the molecular systematics of foraminifera. *Micropaleontology* 46, 1-12.

Pawlowski, J., Swiderski, Z. and Lee, J.J., 1995. Observation on the ultrastructure and reproduction of *Trochammina* sp. (Foraminiferida). In: Kaminsli, M.A., Geroch, S. and Gasinski, M.A. (Editors), Proceedings of the Fourth International Workshop on Agglutinated Foraminifera. Grzybowski Foundation Special Publication, Krakow Poland, pp. 233-237.

Pawlowski, J. and Holzmann, M., 2002. Molecular phylogeny of Foraminifera a review. *European Journal of Protistology* 38 (1), 1-10.

Person-Le Ruyet, J., Mahé, K., Le Bayon, N. and Le Delliou, H., 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, Dicentrarchus labrax. *Aquaculture* 237 (1-4), 269-280.

Phleger, F.B. and Soutar, A., 1973. Production of benthic foraminifera in three east Pacific oxygen minima. *Micropaleontology* 19, 110–115.

Pucci, F., Geslin, E., Barras, C., Morigi, C., Sabbatini, A., Negri, A. and Jorissen, F.J., Submitted. Survival of benthic foraminifera under hypoxic conditions: Results of an experimental study using the CellTracker Green method. *Marine Ecology*.

Qasim, S.Z., 2000. Oceanography of the northern Arabian Sea. *Deep-Sea Research* 29, 1041–1068.

Rathburn, A.E. and Corliss, B.H., 1994. The ecology of living (stained) deep-sea benthic foraminifera from the Sulu Sea. *Paleoceanography* 9 (1), 87-150.

Rathburn, A.E., Corliss, B.H., Tappa, K.D. and Lohmann, K.C., 1996. Comparisons of the ecology and stable isotopic compositions of living (stained) benthic foraminifera from the Sulu and South China Seas. *Deep-Sea Research Part I* 43 (10), 1617-1646.

Rathburn, A.E. and De Deckker, P., 1997. Magnesium and strontium compositions of Recent benthic foraminifera from the Coral Sea, Australia and Prydz Bay, Antarctica. *Marine Micropaleontology* 32 (3-4), 231-248.

Rathmann, S. and Kuhnert, H., 2008. Carbonate ion effect on Mg/Ca, Sr/Ca and stable isotopes on the benthic foraminifera *Oridorsalis umbonatus* off Namibia. *Marine Micropaleontology* 66 (2), 120-133.

Ravelo, A.C. and Hillaire-Marcel, C., 2007. Chapter Eighteen The Use of Oxygen and Carbon Isotopes of Foraminifera in Paleoceanography. In: Hillaire-Marcel, C. and De Vernal, A. (Editors), Paleoceanography of the Late Cenozoic. Developments in Marine Geology. Elsevier, pp. 735-764.

Reichart, G.-J., Jorissen, F., Anschutz, P. and Mason, P.R.D., 2003. Single foraminiferal test chemistry records the marine environment. *Geology* 31 (4), 355-358.

Risgaard-Petersen, N., Langezaal, A.M., Ingvardsen, S., Schmid, M.C., Jetten, M.S.M., Op Den Camp, H.J.M., Derksen, J.W.M., (...) and van der Zwaan, G.J., 2006. Evidence for complete denitrification in a benthic foraminifer. *Nature* 443 (7107), 93-96.

Rixen, T., Haake, B., Ittekkot, V., Guptha, M.V.S., Nair, R.R. and Schlüssel, P., 1996. Coupling between SW monsoon-related surface and deep-ocean processes as discerned from continuous particle flux measurements and correlated satellite data. *Journal of Geophysical Research* 101, 28569–28582.

Rixen, T., Ittekkot, V., Haake-Gaye, B. and Schäfer, P., 2000. The influence of the sw monsoon on the deep-sea organic carbon cycle in the Holocene. *Deep-Sea Research II* 47, 2629–2651.

Rohling, E.J. and Cooke, S., 1999. Stable Oxygen and Carbon Isotopes in Foraminiferal Carbonate Shells. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Publishers, pp. 239-258.

Rosenthal, Y., Boyle, E.A. and Slowey, N., 1997. Temperature control on the incorporation of magnesium, strontium, fluorine, and cadmium into benthic foraminiferal shells from Little Bahama Bank: Prospects for thermocline paleoceanography. *Geochimica et Cosmochimica Acta* 61 (17), 3633-3643.

Round, F.E., Crawford, R.M. and Mann, D.G., 1990. The diatoms: Biology and Morphology of the Genera. Cambridge University Press.

Roy, R.N., Roy, L.N., Vogel, K.M., Porter-Moore, C., Pearson, T., Good, C.E., Millero, F.J. and Campbell, D.M., 1993. The dissociation-constants of carbonic-acid in seawater at salinities 5 to 45 and temperatures 0°C to 45°C. *Marine Chemistry* 44, 249-267.

Russell, A.D., Hönisch, B., Spero, H.J. and Lea, D.W., 2004. Effects of seawater carbonate ion concentration and temperature on shell U, Mg, and Sr in cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta* 68 (21), 4347-4361.

Saraswati, P.K., 2004. Ontogenetic isotopic variation in foraminifera – Implications for palaeo proxy. *Current Science* 86 (6), 858-860.

Sarnthein, M., Thiede, J., Pflaumann, U., Erlenkeuser, H., Fütterer, D., Koopman, B., Lange, H. and Seibold, E., 1982. Atmospheric and oceanic circulation patterns off Northwest Africa during the past 25 million years. In: Von Rad, U., Hinz, K., Sarnthein, M. and Seibold, E. (Editors), Geology of the Norwest African Continental Margin. Springer, Berlin, pp. p. 545-604.

Schmiedl, G., De Bovée, F., Buscail, R., Charriére, B., Hemleben, C., Medernach, L. and Picon, P., 2000. Trophic control of benthic foraminiferal abundance and microhabitat in the bathyal Gulf of Lions, western Mediterranean Sea. *Marine Micropaleontology* 40, 167-188.

Schmiedl, G., Pfeilsticker, M., Hemleben, C. and Mackensen, A., 2004. Environmental and biological effects on the stable isotope composition of recent deep-sea benthic foraminifera from the western Mediterranean Sea. *Marine Micropaleontology*.

Schumacher, S., Jorissen, F.J., Dissard, D., Larkin, K.E. and Gooday, A.J., 2007. Live (Rose Bengal stained) and dead benthic foraminifera from the oxygen minimum zone of the Pakistan continental margin (Arabian Sea). *Marine Micropaleontology* 62 (1), 45-73.

Schweizer, M., Pawlowski, J., Duijnstee, I.A.P., Kouwenhoven, T.J. and van der Zwaan, G.J., 2005. Molecular phylogeny of the foraminiferal genus Uvigerina based on ribosomal DNA sequences. *Marine Micropaleontology* 57 (3-4), 51-67.

Schweizer, M., Pawlowski, J., Kouwenhoven, T.J., Guiard, J. and van der Zwaan, B., 2008. Molecular phylogeny of Rotaliida (Foraminifera) based on complete small subunit rDNA sequences. *Marine Micropaleontology* 66 (3-4), 233-246.

Seaburg, K.G., Parker, B.C., Wharton Jr., R.A. and Simmons Jr., G.M., 1981. Temperature-growth responses of algal isolates from antarctic oases. *Journal of Phycology* 17, 353-36.

Sen Gupta, B.K. and Machain-Castillo, M.L., 1993. Benthic foraminifera in oxygen-poor habitats. *Marine Micropaleontology* 20 (3-4), 183-201.

Sen Gupta, B.K., 1999a. Introduction to Modern Foraminifera. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Publishers, pp. 3-6.

Sen Gupta, B.K., 1999b. Systematics of Modern Foraminifera. In: Sen Gupta, B.K. (Editor), Modern Foraminifera. Kluwer Academic Publishers, pp. 7-36.

Shackleton, N.J., 1974. Attainment of isotopic equilibrium between ocean water and the benthonic foraminifera genus *Uvigerina*: Isotopic changes in the ocean during the last glacial *Colloq. Int. CNRS* 219, 203-209.

Shackleton, N.J., 1977. Carbon-13 in *Uvigerina*: Tropical Rainforest History and the Equatorial Pacific Carbonate Dissolution Cycles. In: A.Malakoff, N.R.A.a. (Editor), The Fate of Fossil Fuel CO_2 in the Oceans. Plenum Press, New-York, pp. 401-427.

Shackleton, N.J. and Opdyke, N.D., 1973. Oxygen isotope and paleomagnetic stratigraphy of equatorial Pacific core V28-238 : oxygen isotope temperature and ice volumes on a 10^5 year and 10^6 year scale. *Quaternary Research* 3, 39-55.

Shirayama, Y., 1984. The abundance of deep sea meiobenthos in the Western Pacific in relation to environmental factors. *Oceanologica Acta* 7 (1), 113-121.

Shönfeld, J., 2001. Benthic foraminifera and pore-water oxygen profiles: a re-assessment of species boundary conditions at the Western Iberian Margin. *Journal of Foraminiferal Research* 31, 86–107.

Smart, C.W., Thomas, E. and Ramsay, A.T.S., 2007. Middle–late Miocene benthic foraminifera in a western equatorial Indian Ocean depth transect: Paleoceanographic implications. *Palaeogeography, Palaeoclimatology, Palaeoecology* 247 (3-4), 402-420.

Smith, S.L., Codispoti, L.A., Morrison, J.M. and Barber, R.T., 1998. The 1994–1996 Arabian Sea Expedition: An integrated, interdisciplinary investigation of the response of the northwestern Indian Ocean to monsoonal forcing. *Deep-Sea Research II* 45, 1905–1915.

Spero, H.J. and Williams, D.F., 1988. Extracting environmental information from planktonic foraminiferal $\delta^{13}C$ data. *Nature* 335 (6192), 717-719.

Spero, H.J., Lerche, I. and Williams, D.F., 1991. Opening the carbon isotope "vital effect" black box, 2: Quantitative model for interpreting foraminiferal carbon isotope data. *Paleoceanography* 6, 639-655.

Spero, H.J. and Lea, D.W., 1996. Experimental determination of stable isotope variability in *Globigerina bulloides*: implications for paleoceanographic reconstructions. *Marine Micropaleontology* 28, 231-246.

Spero, H.J., Bijma, J., Lea, D.W. and Bernis, B.E., 1997. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* 390 (6659), 497-500.

Sprung, M., 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. *Marine Ecology Progress Series* 17, 83-293.

Stouff, V., Lesourd, M. and Debenay, J.-P., 1999a. Laboratory observations on asexual reproduction (schizogony) and ontogeny of *Ammonia tepida* with comments on the life cycle. *Journal of foraminiferal Research* 29 (1), 75-84.

Stouff, V., Geslin, E., Debenay, J.-P. and Lesourd, M., 1999b. Origin of morphological abnormalities in *Ammonia* (foraminifera): Studies in laboratory and natural environments. *Journal of Foraminiferal Research* 29 (2), 152-170.

Stouff, V., Debenay, J.-P. and Lesourd, M., 1999c. Origin of double and multiple tests in benthic foraminifera: Observations in laboratory cultures. *Marine Micropaleontology* 36 (4), 189-204.

Strawn, K., 1961. Growth of largemouth bass fry at various temperatures. *Transactions of the American Fisheries Society* 90 (3), 334–335.

Suhr, S.B., Pond, D.W., Gooday, A.J. and Smith, C.R., 2003. Selective feeding by benthic foraminifera on phytodetritus on the western Antarctic Peninsula shelf: evidence from fatty acid biomarker analysis. *Marine Ecology Progress Series* 262, 153-162.

Szarek, R., Kuhnt, W., Kawamura, H. and Kitazato, H., 2006. Distribution of recent benthic foraminifera on the Sunda Shelf (South China Sea). *Marine Micropaleontology* 61, 171-195.

Takahashi, T., Broecker, W.S. and Bainbridge, A.E., 1981. The alkalinity and total carbon dioxide concentration in the world oceans. In: Bolin, B. (Editor), Carbon cycle Modelling. Wiley, New York, pp. 159-199 and 271-286.

Thunell, R.C., Williams, D.F. and Howell, M., 1987. Atlantic-Mediterranean water exchange during the late Neogene. *Paleoceanography* 2, 661-678.

Toyofuku, T., Kitazato, H., Kawahata, H., Tsuchiya, M. and Nohara, M., 2000. Evaluation of Mg/Ca thermometry in foraminifera: Comparison of experimental results and measurements in nature. *Paleoceanography* 15 (4), 456-464.

Toyofuku, T., de Nooijer, L.J., Yamamoto, H. and Kitazato, H., 2008. Real-time visualization of calcium ion activity in shallow benthic foraminiferal cells using the fluorescent indicator Fluo-3 AM. *Geochemistry, Geophysics, Geosystems* 9 (5), Q05005, doi:10.1029/2007GC001772.

Turner, J.V., 1982. Kinetic fractionation of carbon-13 during calcium carbonate precipitation. *Geochimica et Cosmochimica Acta* 46, 1183-1191.

Urban, E.R. and Langdon, C.J., 1984. Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. *Aquaculture* 38, 277-291.

Urey, H.C., 1947. The thermodynamic properties of isotopic substances. *Journal of the Chemical Society*, 562-581.

Urey, H.C., Lowenstam, H.A., Epstein, S.R. and McKinney, C.R., 1951. Measurements of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark and Southeastern United States. *Bulletin of the Geological Society of America* 62, 399-416.

van Aken, H.M., 2000. Hydrography of the mid-latitude Northeast Atlantic Ocean - II: The intermediate water masses. *Deep-Sea Research I* 47, 789-824.

van der Zwaan, G.J. and Jorissen, F.J., 1991. Biofacial patterns in river-induced shelf anoxia. In: Tyson and Pearson (Editors), Modern and Ancient Continental Shelf Anoxia. Geological Society Special Publication No 58, London, pp. 65-82.

van der Zwaan, G.J., Duijnstee, I.A.P., Den Dulk, M., Ernst, S.R., Jannink, N.T. and Kouwenhoven, T.J., 1999. Benthic foraminifers: proxies or problems? A review of paleoecologial concepts. *Earth-Sciences Reviews* 46, 213-236.

Verhallen, P.J.J.M., 1987. Early development of *Bulimina marginata* in relation to paleoenvironmental changes in the Mediterranean. *Proc. Kon. Ned. Akad. Wetensch. ser. B* 90, 161-180.

Vincent, E., Killingley, J.S. and Berger, W.H., 1981. Stable isotopes in benthic foraminifera from Ontong-Java Plateau, box cores ERDC 112 and 123. *Palaeogeogaphy, Palaeoclimatology, Palaeoecology* 33, 221-230.

Vinot-Bertouille, A.-C. and Duplessy, J.-C., 1973. Individual isotopic fractionation of carbon and oxygen in benthic foraminifera. *Earth and Planetary Science Letters* 18, 247-252.

Wefer, G. and Killingley, J.S., 1980. Growth histories of strombid snails from Bermuda recorded in their O-18 and C-13 profiles. *Marine Biology* 60, 129-135.

Wefer, G. and Berger, W.H., 1991. Isotope paleontology: growth and composition of extant calcareous species. *Marine Geology* 100 (1-4), 207-248.

Wefer, G., Berger, W.H., Bijma, J. and Fischer, G., 1999. Clues to ocean history: A brief overview of proxies. In: Fischer, G. and Wefer, G. (Editors), Use of proxies in paleoceanography: Examples from the South Atlantic. Springer-Verlag, Berlin, Heidelberg, pp. 1-68.

Wheaton, F.W., 1977. Aquacultural Engineering. Wiley, J., New York, 708 pp.

Whyte, J.N.C., Bourne, N. and Hodgson, C.A., 1990. Nutritional condition of rock scallop,Crassadoma gigantea (Gray), larvae fed mixed algal diets. *Aquaculture* 86, 25-40.

Wiggert, J.D., Jones, B.H., Dickey, T.D., Brink, K.H., Weller, R.A., Marra, J. and Codispoti, L.A., 2000. The Northeast Monsoon's impact on mixing, phytoplankton biomass and nutrient cycling in the Arabian Sea. *Deep-Sea Research II* 47, 1353–1385.

Wilson-Finelli, A., Chandler, G.T. and Spero, H.J., 1998. Stable isotope behavior in paleoceanographically important benthic foraminifera: Results from microcosm culture experiments. *Journal of foraminiferal Research* 28 (4), 312-320.

Woodruff, F., Savin, S.M. and Douglas, R.G., 1980. Biological fractionation of oxygen and carbon isotopes by recent benthic foraminifera. *Marine Micropaleontology* 5, 3-11.

Wyrtki, K., 1973. An equatorial jet in the Indian Ocean. *Science* 181 (4096), 262-264.

Zeebe, R.E., 1999. An explanation of the effect of seawater carbonate concentration on foraminiferal oxygen isotopes. *Geochimica et Cosmochimica Acta* 63 (13/14), 2001-2007.

LISTE DES FIGURES, TABLEAUX, PLANCHES ET ANNEXES

LISTE DES FIGURES

CHAPITRE 1 :

CHAPITRE 3 :

CHAPITRE 4 :

CHAPITRE 5 :

LISTE DES TABLEAUX

CHAPITRE 2 :

CHAPITRE 3 :

CHAPITRE 4 :

CHAPITRE 5 :

LISTE DES PLANCHES

CHAPITRE 2 :

CHAPITRE 3 :

CHAPITRE 4 :

LISTE DES ANNEXES

CHAPITRE 2 :

CHAPITRE 3 :

CHAPITRE 5 :

RÉSUMÉ

L'objectif de cette thèse est de définir les conditions expérimentales nécessaires à la culture de foraminifères benthiques profonds dans le but d'affiner la calibration de proxies paléocéanographiques. Jusqu'à présent les études expérimentales sur ces organismes sont rares compte-tenu des difficultés rencontrées pour les maintenir actifs. Notre travail s'est concentré sur l'espèce *Bulimina marginata* (*sensu lato*) qui présente l'intérêt de bien s'adapter aux conditions en laboratoire.

Dans un premier temps, nous avons déterminé les conditions de vie optimale de cette espèce, à savoir les conditions permettant de produire un maximum de juvéniles et d'obtenir leur croissance dans un délai court. Nous avons donc testé, en laboratoire, l'influence de différentes températures (6-14°C) et de différents types de nourriture (algues vertes ou diatomées, fraîche ou lyophilisée) sur la reproduction (délai avant reproduction, quantité de juvéniles produits, etc.) et la croissance (taux de croissance du test) de *B. marginata*.

Nous avons ensuite mis au point deux protocoles expérimentaux permettant d'obtenir des foraminifères ayant calcifié la totalité de leur coquille dans des conditions physico-chimiques stables et contrôlées (température, salinité, chimie des carbonates).

Finalement, nous avons étudié la composition isotopique des tests de foraminifères calcifiés à différentes températures dans ces systèmes stables. L'analyse de tests de différentes tailles a mis en évidence l'effet ontogénétique sur la composition isotopique de l'oxygène et du carbone des foraminifères benthiques profonds. Par la suite, nous avons établi des équations de paléotempérature en fonction des différentes classes de taille étudiées et déterminé les facteurs de correction à appliquer aux valeurs de $\delta^{18}O$ des tests de différentes tailles de *B. marginata* pour obtenir la valeur de la calcite à l'équilibre.

Mots clés : culture, foraminifères benthiques profonds, température, composition isotopique ($\delta^{18}O$, $\delta^{13}C$), *Bulimina marginata*.

ABSTRACT

The purpose of this research is to define the experimental conditions necessary to culture deep-sea benthic foraminifera, so as to refine the calibration of paleoceanographic proxies. Until recently, experimental studies with these organisms have been rare, considering the difficulties encountered to maintain their activity. Our work focuses on *Bulimina marginata* (*sensu lato*) which has the interest to be well adapted to laboratory conditions.

Firstly, we determined the optimal living conditions for this species, namely the conditions which allowed to produce a maximum of juveniles and to promote their growth in a short period of time. Therefore we tested, in the laboratory, the influence of different temperatures (6-14°C) and different food types (green algae or diatoms, fresh or freeze-dried) on the reproduction (time before reproduction event, quantity of produced juveniles, etc.) and growth (shell growth rate) of *B. marginata*.

Next, we set up two experimental protocols in order to obtain foraminifera that calcified the totality of their shell under stable and controlled physico-chemical conditions (temperature, salinity, carbonate chemistry).

Finally, we studied the isotopic composition of foraminiferal shells grown at different temperatures in these stable systems. The analyses of different sizes of shells revealed an ontogenetic effect on the oxygen and carbon isotopic composition of deep-sea benthic foraminifera. Subsequently, we established paleotemperature equations according to the different size fractions studied and we determined correction factors to apply to the $\delta^{18}O$ of *B. marginata* shells of different sizes in order to obtain the equilibrium calcite value.

Key words: culture, deep-sea benthic foraminifera, temperature, isotopic composition ($\delta^{18}O$, $\delta^{13}C$), *Bulimina marginata*.